MECHANISMS OF BLOOD PRESSURE REGULATION IN HUMANS ELUCIDATED BY ALTERATIONS IN GRAVITY AND MAXIMAL EXERCISE

BY

KEITH A. ENGELKE

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1994

To my parents who taught me that with faith, patience, perseverance, and humor, no challenge is too daunting; and to Bridgette.

ACKNOWLEDGEMENTS

I am greatly indebted to the many individuals who have provided friendship, guidance, and support over the past four years. First, I would like to express my sincere appreciation to my primary advisor and friend, Victor A. Convertino, Ph.D., who challenged me to achieve to the highest standard and provided invaluable advice and direction. I am also indebted to my advisory committee co-chairman, Charles E. Wood, Ph.D., who gave me an opportunity to broaden my perspective and encouraged me to strive for excellence. Together, Drs. Convertino and Wood have not only provided outstanding training, but have also set an enviable example of professional responsibility and dedication. My sincere thanks are also extended to the other members of my advisory committee, Scott K. Powers, Ph.D. and Thomas J. Wronski, Ph.D., for their time and support of this project and my career.

I must also thank all the dedicated professionals at NASA-Kennedy Space Center, NASA-Ames Research Center, Humana-Lucerne Hospital, and Brooks Air Force Base who helped make this project a success. I am particularly indebted to the excellent people in the Kennedy Space Center Biomedical Operations and Research Office including Dr. Wycke Hoffler,

Don Doerr, Sandy Reed, Art Maples, Marc Duvoison, Barry Slack, Dick Triandifils, Jill Polet, Mike Merz, Anne Bowers, and Mary Lou Lasley. In addition, I would also like to thank those at Ames Research Center who provided invaluable support including Dee O'Hara, Elizabeth Lowe, and Drs. Joan Vernikos, Charles Wade, and John Greenleaf. I am also indebted to Dr. Craig Crandall for his friendship and many hours of hard work on the Ames bedrest studies and to Dr. Dave Ludwig for assistance with statistical analyses.

An additional thank you goes to all my colleagues, classmates, and professors at the University of Florida including Tim Cudd, Randy Braith, Christine Saoud, Yeoung-Choy Kam, Maria Castro, Melanie Pecins-Thompson, Mike Welsch, Jane Eason, Cathy Golden, and Drs. Maureen Keller-Wood, Wendell Stainsby, and Michael Pollock for their support during this challenging and rewarding adventure.

Finally, I would like to thank the Florida Space Grant Consortium for providing the financial support necessary to allow me to dedicate the totality of my efforts to this endeavor.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS iii

LIST OF TABLES vii

LIST OF FIGURES viii

KEY TO ABBREVIATIONS x

ABSTRACT xiii

CHAPTER 1 INTRODUCTION 1

CHAPTER 2 BACKGROUND REVIEW 5

2.1 A Brief Review of the Baroreflexes 5
2.1.1 Arterial Baroreceptors 5
2.1.2 Cardiopulmonary Baroreceptors 10
2.2 Methods of Testing Baroreflex Functionin Humans 11
2.2.1 Carotid-Cardiac Baroreflex 12
2.2.2 Aortic Baroreflex 13
2.2.3 Cardiopulmonary Baroreflex 14
2.2.4 Integrated Baroreflex Function 15
2.3 Baroreflex Plasticity 16
2.3.1 Baroreflex Resetting 16
2.3.2 Altered Baroreflex Sensitivity 18
2.4 Physiology of Altered Baroreflex Function 19
2.4.1 Blood Volume 19
2.4.2 Hormonal Stimuli 22
2.4.3 Ionic Sensitivity 28
2.4.4 Mechanical Stimuli 28
2.5 Baroreflex Responses to Altered Orthostatic Conditions 29
2.5.1 Anti-Orthostasis (μ-g) 29
2.5.2 Orthostasis (1-g) 32
2.6 Effects of Exercise on Baroreflex Function 39
2.6.1 Endurance Exercise Training 39
2.6.2 Dynamic Maximal Exercise 41
2.7 Summary 44
2.8 Conclusions 45

CHAPTER 3 GENERAL METHODOLOGY 49

3.1 Experimental Design 49
3.2 Experimental Procedures and Protocols 49
3.2.1 Study 1 49

3.2.2 Study 2 50
3.3 Subjects 52
3.4 Measurements and Techniques 53
3.4.1 Heart Rate and Blood Pressure 53
3.4.2 Carotid Baroreflex Function 54
3.4.3 Tests of Orthostatic Responses 56
3.4.4 Blood and Plasma Volume 58
3.4.5 Vasoactive Hormones 60
3.4.6 Forearm Blood Flow 62
3.4.7 Leg Compliance 64
3.4.8 Leg Volume 64
3.4.9 Exercise bout 65
3.4.10 Valsalva Maneuver 66
3.4.11 Statistical Analyses 67

CHAPTER 4 AUTONOMIC FUNCTIONS AND ORTHOSTATIC RESPONSES 24 HOURS AFTER ACUTE INTENSE EXERCISE IN PARAPLEGIC SUBJECTS 70

4.1 Abstract 70
4.2 Introduction 71
4.3 Methods 73
4.4 Results 74
4.5 Discussion 80

CHAPTER 5 EFFECT OF A SINGLE BOUT OF EXHAUSTIVE EXERCISE ON INTEGRATED BAROREFLEX FUNCTION AFTER 16 DAYS OF 6° HEAD-DOWN TILT 90

5.1 Abstract 90
5.2 Introduction 91
5.3 Methods 93
5.4 Results 93
5.5 Discussion 98

CHAPTER 6 MAINTENANCE OF LBNP TOLERANCE 24 HOURS AFTER MAXIMAL EXERCISE FOLLOWING 16 DAYS OF SIMULATED MICROGRAVITY EXPOSURE 106

6.1 Abstract 106
6.2 Introduction 107
6.3 Methods 109
6.4 Results 110
6.5 Discussion 116

CHAPTER 7 SUMMARY 125

7.1 Summary 125
7.2 Conclusions 132

LIST OF REFERENCES 135

BIOGRAPHICAL SKETCH 166

LIST OF TABLES

Table 3.1. Physical characteristics of subjects 52

Table 4.1. Baseline hemodynamic, vasoactive hormone, plasma volume, and vascular resistance values during control and 24 h after exhaustive exercise 75

Table 4.2. Mean hemodynamic responses to the Valsalva maneuver 78

Table 5.1. Comparison of mean heart rate and blood pressure changes to the Valsalva maneuver between experimental conditions 95

Table 6.1. Reproducibility of baseline measures between experimental conditions 110

LIST OF FIGURES

Figure 2.1. Cardiovascular reflex responses to an orthostatic challenge 48

Figure 3.1. Representative mean arterial pressure and heart rate responses to a 15-s Valsalva maneuver at an expiratory pressure of 30 mmHg 68

Figure 4.1. Comparison of forearm vascular resistance in the supine posture and after 15 min of 70° head-up tilt in the control and exercise conditions 76

Figure 4.2. Mean heart rate and systolic blood pressure reponses to the Valsalva maneuver in the control and 24 h post-exercise conditions 77

Figure 4.3. Carotid-cardiac baroreflex stimulus-response relationships, plotted over the range of pressure from which maximum slopes were derived, during control and 24 h after exercise 79

Figure 4.4. Circulating hormone levels during supine baseline and at the end of 70° HUT during control and post-exercise conditions 81

Figure 5.1. Mean HR and MAP responses to a 15-s Valsalva maneuver on day R-1 at an expiratory pressure of 30 mmHg in control and post-exercise conditions 96

Figure 5.2a. Ratio of the unit change in HR to unit change in MAP during phase II of the Valsalva maneuver 97

Figure 5.2b. Change in MAP from pre-Valsalva baseline during late phase II 97

Figure 6.1a. Maximal LBNP tolerance time before and after HDT 112

Figure 6.1b. Difference in LBNP tolerance time from pre-HDT for each of the seven subjects 112

Figure 6.2a. MAP response at peak LBNP 113

Figure 6.2b. Heart rate response at peak LBNP 113

Figure 6.3. Range of forearm vascular responses to LBNP before and after 16 days HDT in control and exercise conditions 114

Figure 6.4. Carotid-cardiac baroreflex stimulus-response relationships before, on day 16, and just prior to re-ambulation from 16 days HDT in the control and exercise conditions 115

Figure 7.2. Reflex responses to orthostasis following microgravity exposure 126

Figure 7.2. Specific components of the orthostasis-stimulus cardiovascular-reflex response cascade which are enhanced following completion of acute, maximally intense exercise. 128

KEY TO ABBREVIATIONS

Ang II	angiotensin II
ANOVA	analysis of variance
ANP	atrial natriuretic peptide
AVP	arginine vasopressin
$beats \cdot mmHg^{-1}$	beats per millimeter of mercury
BP	blood pressure
bpm	beats per minute
C	centigrade
CCB	carotid-cardiac baroreflex
cm	centimeter
CRF	corticotropic releasing hormone
DBP	diastolic blood pressure
E	epinephrine
ECG	electrocardiogram
EDTA	etyylenediaminetetraacetic acid
FVR	forearm vascular resistance
g	gravity
h	hour(s)
HDT	head-down tilt
HR	heart rate
HUT	head-up tilt
kcal	kilocalorie

kg	kilogram
$kpm \cdot m^{-1}$	kilopond meters per minute
$L \cdot min^{-1}$	liters per minute
LBNP	lower body negative pressure
LC	leg comliance
LV	leg volume
MAP	mean arterial pressure
$mEq \cdot day^{-1}$	milli-equivalents per day
min	minute(s)
ml	milliliter
$ml \cdot mmHg^{-1}$	milliliters per millimeter of mercury
mmHg	millimeters of mercury
mo	month(s)
$msec \cdot mmHg^{-1}$	millisecond per millimeter of mercury
NE	norepinephrine
$ng \cdot ml^{-1} \cdot h^{-1}$	nanogram per milliliter per hour
PRA	plasma renin activity
pru	peripheral resistance unit
PV	plasma volume
s	second (s)
SBP	systolic blood pressure
SE	standard error
SNA	sympathetic nerve activity
SV	stroke volume
RIA	radioimmunoassay

rpm	revolutions per minute
VM	Valsalva maneuver
VO_2max	maximum oxygen uptake
yr	year

Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

MECHANISMS OF BLOOD PRESSURE REGULATION IN HUMANS ELUCIDATED BY ALTERATIONS IN GRAVITY AND MAXIMAL EXERCISE

By

Keith A. Engelke

April, 1994

Chairman: Charles E. Wood
Cochairman: Victor A. Convertino
Major Department: Physiology

To test the hypothesis that maximal exercise could enhance the function of mechanisms responsible for blood pressure control, two repeated-measure (cross-over design) experiments were conducted in which heart rate (HR), blood pressure (BP), forearm vascular resistance (FVR), and vasoactive hormone responses were measured during an orthostatic challenge in seventeen volunteers prone to postural hypotension on two occassions: 1) 24 hours post-exercise and 2) without exercise (control). Isolated carotid-cardiac (CCB) and integrated baroreflex sensitivity, plasma volume (PV), and leg vascular responses were also recorded.

Study 1 investigated the effect of maximal exercise on blood pressure stability during 70° head-up tilt (HUT) in 10 paraplegic subjects. During HUT, the reduction in systolic blood pressure (SBP) observed in the control condition was larger than that following exercise. Maintenance of SBP during post-exercise HUT was associated with increased FVR and accentuated baroreflex sensitivity, and was independent of differences in plasma hormone levels, leg compliance, and blood volume.

In Study 2, cardiovascular responses during lower body negative pressure (LBNP) to pre-syncope were measured in 7 subjects following 16 days of 6° head-down tilt (HDT). PV, CCB, and integrated baroreflex sensitivity were also measured. During LBNP 24 hours after exercise, time to pre-syncope was unchanged from pre-HDT levels compared to a 2-min decrease observed in controls. HR was similar in both conditions as was leg volume. Improved blood pressure stability and maintenance of orthostatic tolerance post-exercise was associated with greater FVR, restored PV, and enhanced CCB and integrated baroreflex sensitivity.

Thus, maximal exercise enhanced BP regulation by improving control of vascular resistance and increasing baroreflex sensitivity and plasma volume. These adaptations appear to be effective in ameliorating microgravity-induced orthostatic hypotension.

CHAPTER 1
INTRODUCTION

Compromised blood pressure control, manifested as hypotension during an orthostatic challenge, has been consistently observed following exposure to microgravity and its ground-based analogs (Blomqvist and Stone, 1983; Convertino et al., 1990a, 1991; Greenleaf et al., 1989; Hoffler, 1977; Johnson et al., 1977b; Stegemann et al., 1975). Although not completely understood, an array of mechanisms and their interactions underlying orthostatic hypotension including inadequate venous return and cardiac output due to reduced blood volume (Convertino, 1990, 1991; Greenleaf et al., 1989; Sandler, 1986), attenuated autonomically-mediated cardiovascular reflex responsiveness (Convertino et al., 1990a; Eckberg and Fritsch, 1991, 1992; Fritsch et al., 1992), and increased venous compliance of the lower extremities (Buckey et al., 1992; Convertino et al., 1989b; Hoffler, 1977) have been observed following prolonged spaceflight, bedrest, and chair confinement. These adaptations suggest that gravity has a significant influence on the functional capacity of blood pressure control mechanisms.

Attempts to minimize or prevent orthostatic instability induced by exposure to weightlessness through application of

conventional endurance-type exercise training programs have proven ineffective (Convertino, 1990; Greenleaf et al., 1989). It is likely that this is due to a failure to understand the mechanisms underlying orthostatic hypotension and the way in which they are affected by exercise. Recently, single bouts of intense exercise have been shown to increase plasma volume (Gillen et al., 1991) and sensitivity of the autonomically-mediated carotid-cardiac baroreflex (Convertino and Adams, 1991; Somers et al., 1985) and to decrease the incidence of syncopal episodes following exposure to groundbased simulations of microgravity such as water immersion (Stegemann et al., 1975) and bedrest (Convertino, 1987b). Taken together, these observations suggest acute, maximally intense exercise may provide a specific physiological stimulus that can acutely attenuate or reverse microgravity-induced cardiovascular adaptations associated with orthostatic hypotension.

To better understand the association between mechanisms of blood pressure regulation and microgravity-induced orthostatic hypotension, the two studies included in this dissertation were conducted to examine effects of an exercise stimulus on basic mechanisms associated with blood pressure regulation. Plasma volume, leg vascular responses, arterial baroreflex sensitivity, plasma levels of vasoactive hormones, and cardiovascular responses to changes in intra-thoracic pressure and orthostatic stress were measured with and without exercise treatment in subjects prone to postural

hypotension. The first study (Study 1) was designed to evaluate the effect of a bout of graded exercise on cardiac and vascular responses to orthostasis in subjects whose blood pressure control system had been impaired due to prolonged confinement to wheelchairs. In this study, we tested the hypothesis that maximal exercise would provide blood pressure homeostasis during head-up tilt.

The purpose of the second study (Study 2) was to determine the effects of exhaustive exercise on reversing cardiovascular adaptations associated with orthostatic intolerance following 16 days of 6° head-down tilt. We hypothesized that one exercise bout would be effective in attenuating or reversing decrements in blood volume, baroreflex sensitivity, cardiovascular function, and orthostatic stability following head-down tilt and enhance blood pressure control during lower body negative pressure.

The mechanisms underlying enhanced blood pressure control during recovery from a bout of maximal exercise are unclear. This dissertation attempts to elucidate the contribution of changes in blood volume and alterations in responsiveness of autonomically-mediated baroreflexes to blood pressure maintenance during orthostasis. Therefore, Chapter 2 provides a background review of baroreceptor function and stimuli which alter baroreflex activity. Chapter 3 describes the general experimental design and methodology used in this project. Chapter 4 evaluates the effect of maximal exercise on the blood pressure response to

head-up tilt of subjects permanently confined to the seated posture. Chapter 5 describes the effect of exercise on integrated cardiovascular reflex response to a change in intra-thoracic pressure elicited by the Valsalva maneuver and Chapter 6 examines the influence of a single bout of leg exercise on cardiac and vascular response to lower body negative pressure. Chapter 7 provides a general summary of the two projects and offers overall conclusions. The results of these studies should provide a better understanding of adaptive and integrative processes of components important in blood pressure regulation.

CHAPTER 2
BACKGROUND REVIEW

2.1 A Brief Review of the Baroreflexes

2.1.1 Arterial Baroreceptors

Arterial baroreceptors are mechano-receptors located in the walls of large systemic arteries including the aortic arch, brachiocephalic artery, and carotid sinuses (Boss and Green, 1956; Green, 1954). Afferent signals from the arch of the aorta are transmitted through the left and right aortic (depressor) nerves to the vagus nerve and ultimately to the nucleus tractus solitarius in the medullary area of the brain stem (Boss and Green, 1956; Nakayama, 1965; Nonindez, 1935). Sensory input from the carotid sinus region travels to the nucleus tractus solitarius as well, but via the sinus (Hering's) and glossopharyngeal nerves.

Barosensitive nerve endings are found in areas of the arteries with large quantities of elastic tissue (Muratori, 1967). Approximately 40% of the tissue comprising the walls of the aortic arch is an elastin-collagen mixture (Bader, 1963) which is almost free of smooth muscle (Gregoreva, 1962). Similarly, the carotid sinus is thinner (Adams, 1958; Addison, 1944; Rees, 1968; Rees and Jepson, 1970), contains

less smooth muscle (Addison, 1944; Bagshaw and Fischer, 1971; Muratori, 1967; Rees and Jepson, 1970), and shows a higher elastin content (Addison, 1944; Rees and Jepson, 1970) than other areas of the carotid artery.

Although their name implies a pressure-sensitive quality, baroreceptors are stretch receptors which respond to deformation of the vessel wall in which they are located (Hauss et al., 1949; Angell-James, 1971). There is strong experimental evidence which shows that the degree of wall deformation determines the electrical activity of the carotid sinus and aortic arch baroreceptors. Hauss et al. (1949) demonstrated that the reflex fall of blood pressure produced by an increase in carotid sinus pressure is abolished if the stretching of the carotid artery is prevented by a plaster cast appplied to the outside of the sinus region. Additionally, Angell-James (1971) reported that increased baroreceptor activity produced by elevation of intrathoracic pressure can be prevented by simultaneously increasing the extramural pressure by the same amount. Experiments in man have shown that changing pressure in a chamber surrounding the neck results in reflex changes in heart rate and blood pressure and provide further evidence that altered transmural pressure is the stimulus for baroreceptor activiation (Bevegard and Shepherd, 1966; Ernsting and Parry, 1957).

Koch (1931) was the first to demonstrate that when carotid sinus pressure was changed in a stepwise manner, mean arterial pressure exhibited an inverse sigmoidal response to

the change in intrasinus pressure. These findings were later substantiated by Bronk and Stella (1932) who observed that the impulse frequency in Hering's nerve exhibited a positive sigmoidal relationship to changes in sinus pressure. These early findings identified that baroreceptors can be characterized as having a threshold pressure at which receptor discharge is initiated, a pressure range for which the discharge rate increases with a rise in mean arterial blood pressure, and an asymptotic saturation pressure beyond which there is little increase in baroreceptor activity (Koushanpour, 1991).

The aortic and carotid baroreceptors exhibit different threshold and saturation characteristics. Carotid sinus baroreceptors are silent at arterial pressures between 0 and 60 mmHg, but above 60 mmHg, they respond progressively and reach maximum discharge capacity at approximately 180 mmHg (Koushanpour, 1991). Aortic baroreceptors respond in a manner similar to that of the carotids except that they exhibit a threshold pressure approximately 30 mmHg higher, i.e., 90 mmHg (Koushanpour, 1991). Therefore, in the normal operating range of approximately 100 mmHg (80 to 180 mmHg), slight changes in pressure elicit strong baroreceptor-mediated autonomic reflexes to return arterial pressure to within homeostatic limits.

Effective baroreceptor function is necessary to respond to transient alterations in arterial pressure (Brown, 1980). In the face of increasing pressure, baroreceptor-generated

signals ascend afferent pathways and enter the nucleus tractus solitarius where secondary signals inhibit the medullary vasoconstrictor center and excite the vagal center stimulating vasodilation and decreased myocardial inotropic and chronotropic response (Brown, 1980). These actions lead to lowered peripheral resistance, cardiac output, and ultimately, lower blood pressure. Conversely, a sudden fall in arterial pressure leads to reflex actions which increase cardiac output and sytemic resistance to raise blood pressure.

The bradycardic response to baroreceptor stimulation in humans is mediated through vagal cholinergic mechanisms. Several investigators (Eckberg et al., 1971; Pickering et al., 1972; Simon et al., 1977; Takeshita et al., 1979) have demonstrated that elongation of the R-R interval which accompanied a rise in arterial pressure following administration of phenylephrine was not reduced by propranolol (Jose and Taylor, 1969), but was abolished by atropine. Others have reported similar observations in response to stimulation of the carotid baroreceptors by neck suction (Eckberg, 1977; Eckberg et al., 1976).

In contrast, there is a lack of consensus regarding the autonomic mechanisms mediating the tachycardic response to arterial baroreceptor unloading. It has been observed that the early tachycardia observed after administration of vasodilators was unaffected by propranolol, but abolished by atropine, suggesting a predominant vagal mediation of this

response (Leon et al., 1970; Mancia et al., 1979; Mroczek et al., 1976; Pickering et al., 1972). Contrary to these reports, others have demonstrated that the increase in heart rate produced during infusions of nitroglycerin was reduced by atropine, but could only be abolished by combined administration of atropine and a beta-adrenergic blocker (Goldstein et al., 1975; Robinson et al., 1966). In addition, it has been demonstrated that during lower body negative pressure, tachycardia was diminshed 52% by propranolol with the remaining response abolished by atropine (Bjurstedt et al., 1977). Therefore, it appears there is a significant vagal component to the cardioacceleration which accompanies baroreceptor unloading. However, an increase of sympathetic cardiac influence may contribute to the more sustained component of baroreflex-mediated tachycardia (Mancia and Mark, 1983). Taken together, these results suggest redundancy in mechanisms by which the autonomic nervous system mediates baroreflex-induced tachycardia.

The baroreceptor system markedly reduces daily variation in arterial pressure. This phenomena is readily demonstrated in sinoaortic dennervated animals who exhibit elevated blood pressure and increased blood pressure lability (Cowley et al., 1973). Controversy exists regarding the persistance of this hypertension with some authors arguing it eventually subsides (Guyton et al., 1974) while others suggest elevated blood pressure persists (Scher and Ito, 1978; Alexander, 1979; Touw et al., 1979; Werber and Fink, 1979).

2.1.2 Cardiopulmonary Baroreceptors

The atria (Paintal, 1953) and pulmonary vessels (Coleridge and Kidd, 1963; Ledsome and Kan, 1977) have numerous mechano-receptors in their walls which also play a role in the control of cardiovascular function (Mark and Mancia, 1983). As is the case with arterial baroreceptors, afferent signals from the cardiopulmonary receptors are transmitted to the cardiovascular centers in the medulla via either vagal (Donald and Edis, 1971; Thoren et al., 1976) or sympathetic (Malliani et al., 1975) nerve fibers. Because these receptors are located on the venous (low pressure) side of the circulation, they are not well-suited for detecting small and/or rapid fluctuations in arterial blood pressure. However, information regarding even minor volume changes is provided by these receptors and they elicit reflexes parallel to the arterial baroreflexes to make the total reflex system much more potent for control of blood pressure (Persson, 1991).

Several investigators have demonstrated that low pressure receptors exert an important influence on peripheral vascular resistance during alterations of venous return and cardiac filling pressures in humans. Roddie et al. (1957) observed reflex dilation in skeletal muscle during leg raising. Others (Johnson et al., 1974; Zoller et al., 1977) have noted that as central venous pressure decreased due to

application of low levels (-5 to -20 mmHg) of lower body negative pressure, forearm vascular resistance increased. Low pressure receptors may also mediate alterations in splanchnic resistance (Abboud et al., 1979; Johnson et al., 1974) and venous tone (Abboud and Mark, 1979; Epstein et al., 1968; Samueloff et al., 1966).

Much effort has been expended in an attempt to determine the influence of cardiopulmonary receptors on heart rate. Although this concept, first proposed by Bainbridge (1915) after he observed an increase in heart rate during saline infusions in dogs, is relatively well-supported by data from animal models (Bishop et al., 1976; Gupta, 1975; Vatner et al., 1975;), the existence of such a reflex in humans remains controversial (Doyle et al., 1951; Johnson et al., 1974; Takeshita et al., 1979; Zoller et al., 1972). In contrast, there is compelling evidence to suggest that low pressure baroreceptors participate in the reflex control of renin and vasopressin secretion (Epstein et al., 1981; Epstein et al., 1975; Epstein and Saruta, 1971; Kiowski and Julius, 1978).

2.2 Methods of Testing Baroreflex Function in Humans

Both invasive and non-invasive experimental techniques are employed to assess the function of human baroreceptors. Additionally, there are methods available to isolate specific baroreceptor populations as well as measure integrated baroreflex function. The method by which the reflex function

is analyzed is important because the technique utilized will influence the quantity and quality of the collected data (Mancia and Mark, 1983).

2.2.1 Carotid-Cardiac Baroreflex

In humans, invasive assessment of the carotid baroreflex involves measurement of heart rate and/or blood pressure during direct electrical stimulation of the carotid sinus nerve (Wallin et al., 1975), temporary interruption of nerve traffic with anesthesia (Guz et al., 1966), or permanent denervation via surgical ablation (Angell-James and Lumley, 1964). However, there are several limitations to these techniques including concerns regarding completeness of the section or blockade, non-selectivity of anesthesia, and unanticipated side effects due to the anesthetic or surgical procedure utilized. Thus, these approaches are rarely used in the research laboratory.

A simple, non-invasive method to assess carotid baroreceptor function involves measurement of heart rate and blood pressure during carotid massage (Roddie and Shepherd, 1957). While this method is widely used clinically, it is of little use as a research tool due to the inability to quantify or repeat the stimulus presented to the baroreceptors. Carotid baroreceptor function is also assessed by recording changes in heart rate or blood pressure while carotid distending pressure is altered by a device

which transmits pressure and/or suction to the area surrounding the carotid sinuses (Ernsting and Parry, 1957; Stegemann et al., 1974). Recent advances in this technique allows rapid changes in neck pressure and suction to be delivered to the anterior 2/3 of the neck for 10-15 seconds (Eckberg and Eckberg, 1982; Sprenkle et al., 1986). The carotid-cardiac baroreflex stimulus-response curve is calculated by plotting changes in R-R interval (or blood pressure) against estimated carotid sinus pressure (Sprenkle et al., 1986; Ludwig and Convertino, 1991). The primary criticism of this method is the inability to quantify the exact amount of pressure and/or suction transmitted through the tissue of the neck to the carotid sinus. Despite the work of Ludbrook et al. (1977) who determined that 86% of the applied pressure and 64% of the suction is transmitted directly to the sinuses, it is likely that variability exists in the amount of pressure/suction reaching the sinuses due to differences in body composition of subjects and design of the neck collar device used by investigators.

2.2.2 Aortic Baroreflex

The currently accepted method to assess the isolated aortic baroreflex in humans requires activation of the three baroreceptor populations followed by manipulations which subsequently attenuate or abolish their influence (Ferguson et al., 1985; Sanders et al., 1988). Briefly, the carotid

sinus, aortic, and cardiopulmonary baroreceptors are activated via a phenylephrine-induced increase in arterial pressure. During this increase of arterial pressure, carotid sinus baroreceptor activity is countered by application of neck pressure at a level equal to the elevation of blood pressure. Increased cardiopulmonary baroreceptor activity is negated by lower body negative pressure. Therefore, with the carotid sinus and cardiopulmonary baroreceptor activities clamped at baseline levels, changes in heart rate can be attributed to the aortic baroreceptors. Sensitivity of the aortic baroreflex is then assessed by calculating the ratio of the change in heart rate to the change in mean arterial pressure.

2.2.3 Cardiopulmonary Baroreflex

Cardiopulmonary baroreceptor function is evaluated by examining reflex changes in peripheral vascular conductance stimulated by changes in central venous pressure. Typically, techniques which alter venous return and atrial filling are utilized including lower body positive (Bevegard et al., 1977a) and negative (Brown et al., 1966; Stevens and Lamb, 1965; Wolthuis et al., 1974) pressure, leg elevation (Roddie and Shepherd, 1956; Roddie and Shepherd, 1957), head-out water immersion (Gauer and Henry, 1963; Epstein, 1976), tilt (Roddie and Shepherd, 1957), and respiratory maneuvers (Roddie and Shepherd, 1958; Roddie et al., 1957). Reflex

changes in peripheral blood flow are then measured, most simply via plethysmography.

Lower body negative pressure (LBNP) studies have contributed greatly to the understanding of the role of cardiopulmonary receptors in man. The primary advantage of this techique is that the magnitude of the stimulus can be controlled. Negative pressure of less than -20 mmHg decreases cardiac filling pressure without detectable changes in arterial pulse pressure, mean pressure, arterial dt/dP, or heart rate. Thus, mild lower body negative pressure minimizes the stimulus to low-pressure receptors without significant changes to determinants of arterial (high pressure) baroreceptor activity (Johnson et al., 1974; Zoller et al., 1972).

2.2.4 Integrated Baroreflex Function

Global baroreflex function has been assessed by a variety of methods. However, because these techniques elicit a simultaneous response from many or all baroreceptor populations, conclusions cannot be made regarding the stimulus-response relationship of a specific baroreflex arc. The most common technique to assess integrated baroreflex function involves measuring heart rate in the presence of either a pressor or depressor agent and then calculating the ratio of the change in heart rate to the change in blood pressure (Mancia and Mark, 1983). The slope of this

relationship is indicative of baroreflex sensitivity. A limitation of this method is that only baroreflex control of heart rate can be assessed since vasoactive drugs are administered. Other techniques which cause a change in heart rate and blood pressure such as lower body negative pressure (Smith et al., 1988), head-up tilt (Smith et al., 1987a), and the Valsalva maneuver (Smith et al., 1987b) have also been used to assses global baroreflex function.

2.3 Baroreflex Plasticity

2.3.1 Baroreceptor Resetting

Both human and animal studies have demonstrated that the operating range of baroreflexes is labile. Persistent alterations of mean arterial pressure result in a relative shift of the pressure-stimulus baroreceptor-response curve along the stimulus (pressure) axis in the direction of increased or decreased pressure without a change in the slope (sensitivity or gain) of the steepest part of the curve (Hayward et al, 1993; Igler et al., 1981; Munch and Brown, 1985; Sleight et al., 1977). This change in baroreflex function is referred to as resetting. McCubbin et al. (1956) was the first to demonstrate this phenomenon. Using electroneurographic techniques to record carotid and aortic baroreceptor activity in renal hypertensive dogs, they demonstrated that when the baroreceptors were exposed to

prolonged increases of arterial pressure, the threshold and operating range of the reflex were displaced to a higher point on the pressure-stimulus baroreceptor-firing curve (McCubbin et al., 1956). Subsequently, resetting has been demonstrated in a variety of species and preparations (Dorward et al., 1982; Kreiger, 1970; Kunze, 1981; Salgado and Kreiger, 1978; Sleight et al., 1977). Alterations in reflex function act to maintain, rather than oppose, the prevailing pressure (McCubbin et al., 1956) possibly by influencing the control of heart rate (Chen et al., 1982; Dorward et al., 1985; Fritsch et al., 1989), arterial pressure (Kunze, 1981; Tan and Zucker, 1989), and/or sympathetic nerve activity (Dorward et al, 1985; Heesch and Carey, 1987; Undesser et al., 1975).

In early studies, resetting was reported to occur over hours or days. However, recent investigations have demonstrated a more rapid resetting of baroreceptors. Although the time course of resetting varies due to differences in experimental preparations, baroreceptor activity generally reaches a new steady-state within 5-20 minutes (Mifflin and Kunze, 1982; Munch et al., 1983). Within this time, the entire pressure-baroreceptor activity relationship shifts toward the new prevailing pressure (Coleridge et al., 1981; Coleridge et al., 1984; Dorward et al., 1982; Heesch et al., 1984a; Heesch et al., 1984b; Munch et al., 1983; Munch and Brown, 1985; Undesser et al., 1984). This resetting of baroreceptors allows them to respond

effectively to changes in arterial pressure and buffer such fluctuations.

2.3.2 Altered Baroreflex Sensitivity

In contrast to the frequently observed baroreceptor resetting following exposure to static (Heesch et al., 1984a; Heesch et al., 1984b; Mifflin and Kunze, 1982; Munch et al., 1983; Munch and Brown, 1985) or sinusoidal (Kunze, 1981) elevations in arterial pressure, resetting appears to be markedly attenuated or absent when pressure changes are pulsatile in nature. In support of this hypothesis, Chapleau et al. (1987) have demonstrated that when carotid sinus pressure is low, pulsations increase baroreceptor activity without resetting of the baroreceptor stimulus-response function. Similarly, when Mendelowitz and Scher (1988) exposed a carotid sinus preparation to elevated pulsatile pressures, the resulting blood pressure was lower throughout the period of pulsation compared to that observed when the carotid sinus was exposed to a static pressure profile. Chapleau et al. (1987) also noted that increased baroreflex sensitivity persisted for several minutes after cessation of the pulsing period. Furthermore, they reported that the magnitude of the effect was directly related to duration of the pulsing period and to frequency and amplitude of the pressure pulses (Chapleau et al., 1987).

A complete explanation for these observations remains to be found. It appears that increases in static pressure elicits resetting of the baroreflex possibly reflecting a decreased responiveness of the medullary barosensitive neurons to sustained baroreceptor discharge. In contrast, phasic afferent activity which accompanies pulsatile increases in mean arterial pressure may minimize central adaptation to continued baroreceptor discharge. As a result, there is a greater reflex inhibition of sympathetic activity and subsequent maintenance or enhancement of baroreflex activity to pulsatile pressure increases (Chapleau et al., 1987).

2.4 Physiology of Altered Baroreflex Function

2.4.1 Blood Volume

Several investigators have observed that alteration or redistribution of central blood volume is associated with changes in baroreflex responses (Bevegard et al., 1977b; Billman et al., 1981; Harrison et al., 1986; Mack et al., 1987). Experiments in rats have demonstrated that hypervolemia decreased the blood pressure response to bilateral carotid occlusion, while a moderate hypovolemia tended to increase the blood pressure response to this same stimulus (Castenfors and Sjostrand, 1973). Vatner et al. (1975) noted that elevated mean arterial pressure stimulated

by acute volume loading in conscious dogs elicited a decreased heart rate response and concluded that the sensitivity of the arterial baroreceptor reflex had been reduced. A similar conclusion was reached by others who observed nearly identical results in rabbits (Lubrook and Graham, 1984; Stinnett et al., 1976), dogs (Wang et al., 1992; Dujardin, 1980), and non-human primates (Billman et al, 1981; Cornish et al., 1989). Using human subjects, Pickering et al. (1971) altered effective circulating volume with upright tilt and observed a diminishment of baroreceptor sensitivity compared to supine control. Similarly, Harrison et al. (1986) used head-up and head-down tilt to acutely alter central blood volume and noted that the heart rate response to neck suction was greater in the head-down position. Blood volume may also influence the function of the cardiopulmonary baroreflex (Mack et al., 1987; Thompson et al., 1990).

Conversely, Eckberg et al. (1972, 1976) compared baroreflex sensitivity in the upright and supine postures and detected no difference between conditions. Consistent with these data is the observation that increasing central blood volume by aplying lower body positive pressure (Bevegard et al., 1977a) or by raising the lower limbs and trunk (Takeshita et al., 1979), has no effect on arterial baroreceptor responsiveness. Similarly, Thompson et al. (1990) reported that acute alterations in plasma volume had no influence on the carotid-cardiac baroreflex sensitivity.

Decreasing central blood volume by applying non-hypotensive (-10 to -40 mmHg) lower body negative pressure also failed to alter baroreceptor control of heart rate (Takeshita et al., 1979), but did augment carotid baroreflex control of vascular resistance (Bevegard et al., 1977a; Mancia and Mark, 1983).

An explanation of these discrepancies remains elusive, but it appears that they can be partly attributed to species differences and dissimilarities among experimental techniques. Of particular importance is the magnitude of blood volume alteration induced by each experimental approach (Barajanzi and Cornish, 1987). It is well-established that changes in blood volume alter the activity of mechanoreceptors in the atria and vena cava (Thorn et al., 1976). Reflexes from cardiopulmonary receptors also modulate arterial baroreceptor function (Ludbrook and Graham, 1984). Takeshita et al. (1979) suggested that, in humans, variations of central venous pressure and cardiopulmonary activity within a certain physiological range do not influence arterial baroreceptor control of sinus node activity. Therefore, it is possible that circulating volume must be altered above or below a threshold before high pressure baroreceptor function is influenced (Thompson et al., 1990).

2.4.2 Hormonal Stimuli

Circulating hormones such as norepinephrine (NE), arginine vasopressin (AVP), and angiotensin II (Ang II), in

addition to actions on vascular smooth muscle and fluid regulation, may act in the central nervous system to modulate the regulation of peripheral sympathetic nerve activity (SNA) possibly through a baroreflex-dependent mechanism (Bishop et al., 1987; Ferario et al., 1987). In addition to NE, Ang II, and AVP, atrial natriuretic peptide (ANP), insulin, and corticotropin releasing factor (CRF) may also influence arterial baroreflex control of arterial blood pressure.

Norepinephrine. The proximity of baroreceptor afferents and sympathetic efferents in the aortic arch and carotid sinus regions provides an anatomical basis for sympathetic efferent modulation of baroreceptor activity (Rees, 1967; Reis and Fuxe, 1968; Bock and Gorgas, 1976). Activation of sinoaortic sympathetic nerves via electrical stimulation or topical application of NE alters baroreceptor discharge and produces reflex changes in arterial blood pressure. For example, painting concentrated solutions of NE on the carotid sinus lowers blood pressure (Kirchheim, 1976). Likewise, activating the sinus efferent nerves reduces blood pressure (Kedzi, 1954) and attenuates the response to carotid occlusion (Wurster and Trobiani, 1973).

Although these reports suggest NE modifies baroreceptor activity, what is not fully understood is the mechanism by which these changes occur (Munch et al., 1987). It is unclear if NE acts indirectly via contraction of local vascular smooth muscle or directly through activation of adrenergic receptors on the barosensitive nerve endings.

Both neurogenic and exogenic NE can alter mechanical properties of the arterial wall smooth muscle in which the nerve endings are embedded (Aars, 1971; Aars, 1981; Bagshaw and Peterson, 1967). This hypothesis is also supported by Munch and Brown (1985) who demonstrated that NE constricted rat aortic arch and subsequently decreased activity of the baroreceptors. In contrast, it has also been observed that NE enhances baroreceptor activity independent of changes in smooth muscle tone (Goldman and Saum, 1984; Kunze et al., 1984). Thus, it appears that NE may have two modes of action which lead to altered baroreceptor discharge characteristics.

Angiotensin II. Inappropriate heart rate responses to Ang II-induced increases in arterial pressure as well as similar increases in arterial pressure in intact and sinoaortic denervated dogs during infusions of Ang II have led investigators to conclude that this agent alters baroreflex response (Guo and Abboud, 1984; Matsukawa and Reid, 1990; Reid, 1984). Additionally, it has been observed that there is a reduction in both arterial baroreflex inhibition of sympathetic nerve activity and overall vagal efferent activity during systemic administration of Ang II (Guo and Abboud, 1984; Lumbers et al., 1979) suggesting that Ang II resets the baroreflex operating range toward higher pressures (Matsukawa and Reid, 1990).

The mechanisms by which Ang II acts to reset the arterial baroreflexes remain unclear (Reid, 1984). Angiotensin II may act centrally to reset the operating point

of the arterial baroreflex or to decrease the sensitivity of this relationship (Reid, 1984). In the non-human primate, infusions of Ang II decreased the sensitivity of the cardiac baroreflex during changes in arterial pressure (Garner et al., 1987). This observation has been substantiated by others (Guo and Abboud, 1984; Lee and Lumbers, 1981). Conversely, studies in dogs and rabbits failed to detect any alteration in the slope of the mean arterial pressure-heart rate relationship during changes in arterial pressure (Brooks and Reid, 1986; Matsumura et al., 1989). Interestingly, resetting of the reflex was prevented in rabbits and rats with lesions in the area postrema (Fink et al., 1980) suggesting that Ang II may modify baroreceptor afferent input, and thus baroreceptor activity, in this region of the medulla.

Vasopressin. Studies in humans, dogs, and rabbits have shown that increased circulating arginine vasopressin may act in the central nervous system to augment the sympathoinhibitory influence of the arterial and cardiopulmonary baroreflexes (DiCarlo et al., 1989; Floras et al., 1987; Hasser et al., 1987). This effect has been directly determined by measuring sympathetic nerve activity during intravenous infusions of AVP. In both the anesthetized and conscious rabbit, progressive infusions of AVP produced large decreases in lumbar and renal SNA prior to any detectable increase in arterial pressure (Undesser et al., 1985). When the arterial and cardiopulmonary

baroreflexes had been rendered non-functional by anesthesia or surgery, increased circulating AVP had no effect on renal SNA, suggesting that inhibition of renal SNA during AVP infusion is due to a facilitation of the arterial baroreflex (Undesser et al., 1985).

The mechanism of action of AVP on baroreceptor function remains to be elucidated. Baroreceptor-mediated bradycardia was prevented by blocking the vascular AVP receptor with a V_1 receptor antagonist (Bishop et al., 1987). Ablation of the area postrema returned the slope of the mean arterial pressure-heart rate relationship during AVP infusion to that observed with phenylephrine infusion (Undesser et al., 1985) confirming the hypothesis that AVP acts at the area postrema to modify baroreflex responsiveness. Subsequent studies have shown that endogenous increases in AVP due to osmotic and reflex stimuli facilitate the arterial and cardiopulmonary reflexes (DiCarlo et al., 1989; Hasser et al., 1988). Therefore, it appears reasonable to conclude that AVP plays a role in regulation of arterial baroreflexes, possibly by regulating the operating point of the reflex via actions in the brainstem.

Atrial Natriuretic Peptide. Atrial natriuretic peptide is released into the plasma in response to increased atrial stretch following blood volume expansion (Ballermann and Brenner, 1986). Although this peptide hormone primarily influences salt and water balance, pharmacological concentrations of ANP decreased resting sympathetic nerve

activity (Imaizumi et al., 1987; Niijima, 1989) and shifted the baroreflex curve to a lower arterial pressure range (TerKonda and Bishop, 1987). These findings are supported by other investigators who have shown reflex tachycardia to ANP-induced reductions in arterial pressure to be reduced (Ackermann et al., 1988; Ferrari et al., 1990), although this effect may be somewhat variable (Marks et al., 1990; Volpe et al., 1987). Decreases in heart rate to phenylephrine or volume expansion are enhanced by the presence of ANP (Ferrari et al., 1990; Marks et al., 1990; Volpe et al., 1987). Ebert and Cowley (1988) infused ANP into human subjects and observed that cardioacceleration during neck pressure is attentuated without a change in resting blood pressure or heart rate, while others (Imam et al., 1989) have shown that changes in plasma norepinephrine to lower body negative pressure are reduced in the presence of ANP. These findings are consistent with the observation of Floras (1990) who noted attenuated SNA activity in the presence of ANP in response to reductions in central venous pressure and a cold pressor test.

The mechanisms responsible for these observations remain questionable. The observation that ANP attenuates sympathetic nerve activity following reductions in central venous pressure (Floras, 1990) suggests that afferent cardiopulmonary baroreceptor input may be altered. Alternatively, ANP may modulate central nervous system processing of afferent information resulting in alteration of

baroreflex activation threshold (Bishop et al., 1991). However, other mechanisms may also be involved as illustrated by the observation that the effects of vagal stimulation are augmented by ANP and the reduction in heart rate induced by ANP is blocked by atropine (Ackermann et al., 1988; Atchison and Ackermann, 1990). These findings suggest that ANP influences parasympathetic control of arterial pressure. In addition, ANP interferes with release of norepinephrine from the nerve terminal (Nakamaru and Inagami, 1986).

Other Hormones. Other hormones have been suggested to alter sympathetic nerve activity and thereby influence baroreflex function. Increases in insulin elevate SNA (Morgan et al., 1990) possibly through a central action (Sakaguchi et al., 1988). Little is known about the direct effects of insulin on the function of the baroreflexes, although Hilsted (1982) observed that animals and humans with impaired insulin release have reduced baroreflex function. However, this finding may be associated with peripheral neuropathy (Hilsted, 1982).

Several studies have suggested that hormones associated with the pituitary-adrenal axis regulate sympathetic activity and affect baroreflex function. Glucocorticoids act centrally to suppress sympathetic outflow (Brown and Fisher, 1986), while CRF increases sympathetic outflow as well as release of norepinephrine and epinephrine into the plasma (Fisher and Brown, 1984). CRF also interferes with the arterial baroreflexes, reducing the gain and range of the

reflex curve and increasing the midpoint of the pressure range (Fisher, 1989).

2.4.3 Ionic Sensitivity

Hodgkin and Katz (1949) theorized that reduced extracellular sodium ion concentration could lead to a change in the sensitivity of baroreceptors to suprathreshold pressures. Indeed, Kunze and Brown (1978) and Kunze et al. (1977) have demonstrated that lowering extracellular sodium concentration elevates the operating point, increases the minimum pressure at which the carotid sinus reflex is elicited, and reduces sensitivity of the reflex. Baroreceptors are also calcium and potassium-sensitive and there is evidence to suggest that sodium, calcium, and potassium interact to influence baroreceptor activity (Brown, 1980). However, despite evidence supporting the hypothesis that small changes in electrolyte concentrations alter baroreflex function, there is little understanding of the underlying mechanisms of action (Brown, 1980).

2.4.4 Mechanical Stimuli

There is general agreement that baroreceptors exhibit plasticity in response to persistent mechanical stimuli, possibly due to relaxation of the viscoelastic medium which couples receptor elements to external stimuli (Hubbard, 1958; Lippold et al., 1960). However, interpretation of these data

is made difficult because investigators have exposed baroreceptors to a variety of stimuli including highly damped, sinusoidal, and static pressure profiles in an attempt to elicit alterations in reflex function (Coleridge et al., 1981; Coleridge et al., 1984; Heesch et al, 1984a; Heesch et al, 1984b; Kunze, 1981; Mifflin et al., 1982; Munch et al., 1983; Munch and Brown, 1985; Tan and Zucker, 1989). Regardless, it appears the rate and degree of adaptation is proportional to the intensity of the intravascular pressure stimulus (Eckberg, 1977).

2.5 Baroreflex Responses to Altered Orthostatic Conditions

2.5.1 Anti-Orthostasis (μ-g)

True Microgravity. Only limited physiological data on the effects of true microgravity were collected during the early phases of the US and Russian manned spaceflight programs (Pestov and Geratewohl, 1975). More extensive studies were made during the American Apollo, Skylab, and Space Shuttle missions and the Russian Salyut and Soyuz flights (Blomqvist and Stone, 1983). In addition, earth's gravitational field has been cancelled for up to 45 seconds in aircraft following parabolic flight patterns (Sandler, 1979). Therefore, although data obtained during actual weightlessness are crucial in the study of effects of gravity on cardiovascular function, results must be interpreted with

caution due to the small number of subjects that can be studied in true weightlessness and the limited time of exposure and range of methods available (Blomqvist and Stone, 1983).

Only one study has assessed the baroreflex control of heart rate following exposure to real microgravity. Using a neck collar device, the carotid stimulus-cardiac response relationship was measured before and after 4-5 days of spaceflight (Fritsch et al., 1992). The results indicated that baseline R-R interval was reduced following flight suggesting tachycardia at rest. Additionally, there was a tendency for the slope of the estimated carotid sinus pressure-heart rate relationship to be decreased post-flight. Despite the large variation of response in a relatively small number of subjects, the investigators concluded that the carotid-cardiac baroreflex function was impaired following 4-5 days of microgravity exposure (Fritsch et al., 1992).

Ground-Based Analogs. Technical and economic constraints have hindered the conduction of rigorously controlled experiments during actual microgravity exposure. Consequently, a number of ground-based analogs utilizing both animal and human subjects have been developed which elicit adaptations in the cardiovascular system similar to those observed in true microgravity. However, conclusions drawn from ground-based simulations may be limited due to the presence of residual hydrostatic gradients. Regardless, there is no conclusive evidence that zero gravity has any

unique or specific effects on the cardiovascular system that cannot be reproduced during earth-bound simulations (Blomqvist and Stone, 1983).

Changes in baroreflex sensitivity in human subjects has been assessed using the 6° head-down tilt bedrest and prolonged chair confinement models of microgravity (Convertino et al., 1990a, 1991, 1992; Eckberg and Fritsch, 1992). Both Convertino et al. (1990a, 1991, 1992) and Eckberg and Fritsch (1992) have determined carotid-cardiac baroreflex gain of humans using the neck pressure/neck suction technique. During a 30-day bedrest study, Convertino et al. (1990a) noted a significant reduction in maximal slope of the carotid-cardiac baroreflex at bedrest day 12 which persisted until day 30. These observations are corroborated by data collected during 10 days of head-down tilt bedrest (Eckberg and Fritsch, 1992) and 4-5 days of Space Shuttle flight (Fritsch et al., 1992). In both cases, there was a tendency for a progressive reduction in maximal slope of the carotid-cardiac baroreflex response over the course of microgravity exposure. Interestingly, although data from Convertino et al. (1990a) and Eckberg and Fritsch (1992) verify that simulated microgravity elicits changes in baroreflex gain similar to those noted following spaceflight (Fritsch et al., 1992), the time course of change appears to be different. Bedrest-induced reductions of baroreflex gain occurred after 9-12 days (Convertino et al., 1990a; Eckberg and Fritsch, 1992) whereas true microgravity apparently

elicited a reduction in reflex sensitivity after only 4-5 days (Fritsch et al., 1992).

Prolonged confinement to the seated posture has also been used to investigate the effects of partial gravity on baroreflex sensitivity. In these studies, baroreflex desensitization appears to have occurred despite the presence of a gravity vector along the long axis of the body and its concomitant hydrostatic influence (Blomqvist and Stone, 1983). Convertino et al. (1991) measured vagally-mediated carotid-cardiac baroreflex sensitivity in quadriplegic subjects confined to wheelchairs for an average of 35 months and reported that the stimulus-response relationship of this reflex was decreased compared to that of age and weigh-matched ambulatory controls. Similarly, it appears that baroreflex function may have been compromised in healthy subjects immobilized in the sitting posture for four to ten days as suggested by a smaller increase in heart rate for a given drop in blood pressure, i.e., decreased $\Delta HR/\Delta MAP$, and increased incidence of hypotension during standing following prolonged chair confinement (Lamb et al., 1964; Lamb et al., 1965). Russian investigators have found similar responses (Ioffe, 1968).

2.5.2 Orthostasis (1-g)

The term "orthostasis" is derived from the greek words "ortho", meaning "caused by erect posture" and "stasis",

meaning "stationary" and can therefore describe responses to any perturbation that produces effects similar to those induced by the upright posture (Convertino, 1987a). The primary hemodynamic effect of orthostasis is translocation of blood from the central veins and heart to large veins in the lower extremities caused by gravity or similar stimulus (Blomqvist and Stone, 1983). "Orthostatic intolerance" refers to the inability of cardiovascular reflexes to maintain arterial pressure for adequate cerebral prefusion due to accumulation of blood in the lower extremities (Convertino, 1987a). Since mean arterial blood pressure is the product of heart rate, stroke volume, and peripheral resistance, changes in the physiological condition which affects one of these variables, without appropriate compensation from the others, will lead to an alteration in mean arterial pressure. If the change is severe enough to reduce cerebral perfusion, orthostatic intolerance and syncope may occur.

The incidence of orthostatic intolerance and syncope during lower body negative pressure, standing, or head-up tilt following exposure to both simulated and actual microgravity is well documented (Berry, 1976; Blomqvist and Stone, 1983; Bungo et al., 1985; Convertino et al., 1990a; Greenleaf et al., 1989; Stegemann et al., 1975). Although the array of mechanisms which cause orthostatic hypotension are not entirely understood, inability to maintain blood pressure during orthostasis appears to be a result of an inadequate venous return due to reduced blood volume

(Blomqvist and Stone, 1983), depressed stimulus-response relationship of the carotid-cardiac baroreflex (Blomqvist and Stone, 1983; Convertino et al., 1990a, 1992; Eckberg and Fritsch, 1992), altered cardiopulmonary (Convertino et al., 1994a) and aortic (Crandall et al., 1993) baroreflexes, and altered autonomic function (Eckberg and Fritsch, 1991). Additionally, increased venous compliance of the lower extremities (Buckey et al., 1992; Convertino et al., 1989a, 1989b; Convertino, 1994; Hoffler, 1977) and decreased cardiac output due to diminished vascular volume (Blomqvist and Stone, 1983; Greenleaf et al., 1989; Sandler, 1986) may also contribute to reductions in orthostatic tolerance following exposure to actual or simulated microgravity.

Increased heart rate during a provocative orthostatic test has been the earliest and most consistently observed alteration in cardiovascular function following microgravity exposure (Hyatt, 1971; McCally and Wunder, 1971; McCally et al., 1966, 1968; Miller et al., 1964b; Stevens et al., 1966). Higher heart rates and lower stroke volumes for a given level of orthostatic stress may not be indicative of a lower orthostatic tolerance (Harper and Lyles, 1988; Hyatt et al., 1969). Miller et al. (1964a) observed significantly elevated heart rates during 30-min of 90° head-up tilt after bedrest, but only 42% of subjects actually exhibited pre-syncopal signs or symptoms. Fortney et al. (1991) determined tolerance (defined as the level of LBNP tolerated without symptoms of syncope) after 13 days of head-down bedrest and

reported that, although all subjects had elevated heart rates during LBNP after bedrest, only half had a reduction in the level of negative pressure tolerated. Thus, elevated heart rate may be a compensatory response to gravitational stress, but it does not necessarily indicate reduced tolerance.

The cardiovascular reflex response to an orthostatic challenge is largely dependent on the magnitude of the volume shift induced by experimental perurbation (Blomqvist and Stone, 1983). Therefore, it is important that experimental procedures which elicit a reproducible and quantifiable volume redistribution are employed to study mechanisms underlying cardiovascular responses to orthostasis (Blomqvist and Stone, 1983). These techniques include upright tilt, passive standing, and lower body negative pressure.

Head-Up Tilt. Head-up tilt involves rapidly elevating a table to a position where the head is higher than the feet while the subject is supported by a saddle or harness that allows the legs to hang passively. This technique minmizes muscular activity and provides a relatively reproducible orthostatic stimulus. However, one limitation to this technique is that head-up tilting can only produce a maximal gravitational stimulus of 1-g (Convertino, 1987a).

The hemodynamic responses to varying degrees of head-up tilt have been reported by several investigators. Essentially, as subject is progressively shifted from the supine to upright posture, blood pressure remains stable or increases slightly, heart rate increases, and stroke volume

and cardiac output fall (Blomqvist and Stone, 1983; Hyatt, 1971; Melada et al., 1975; Poliner et al., 1980). Physiologic responses to head-up tilt are altered after exposure to microgravity (Blomqvist et al., 1980; Butler et al., 1991; Greenleaf et al., 1985; Greenleaf et al., 1989; Hoffler, 1977; McCally et al., 1966; Vogt, 1967). Hoffler (1977) subjected 18 Gemini astronauts to 25-min of 70° head-up tilt and noted that supine resting and tilt-induced heart rates increased an average of 29% and 51%, respectively after flight. In addition, there was a 70% increase in leg volume and a marked narrowing of the pulse pressure during post-flight tilting (Hoffler, 1977). Similarly, Vogt (1967) reported greater increases in heart rate and a faster decline in blood pressure during tilt after 12-h of recumbency compared to control and Miller et al. (1964a) observed significantly elevated heart rates during 30-min 90° head-up tilt after bedrest. In general, maximal heart rate during 70° head-up tilt is increased 15-35 bpm before, and 20-60 bpm following, bedrest (Chobanian et al., 1974; Hyatt, 1971; Lancaster and Triebwasser, 1971; Thorton and Hoffler, 1977; Vogt, 1967).

Passive Standing. Passive standing is a widely used technique to evaluate the cardiovascular responses to orthostasis (Blomqvist and Stone, 1983). Although economical and easy to conduct, this method is less than ideal because it promotes muscle contraction in the legs which have the potential to reduce pooling of blood in the lower extremities

and increase venous return, thus enhancing atrial filling and cardiac output. Such muscle contractions are impossible to control from subject to subject during any given test protocol (Blomqvist and Stone, 1983). Regardless, changes in heart rate to this procedure are frequently used as an index of the cardiovascular response to an orthostatic stress before and after microgravity exposure. In general, increases in heart rate average 35 bpm during 5-min stand tests before bedrest (Hyatt et al., 1975; Lancaster and Triebwasser, 1971) and nearly 70 bpm after two weeks of bedrest (Harper and Lyles, 1988). Furthermore, the fall in stroke volume and cardiac output are about twice as great (Harper and Lyles, 1988). Interestingly, Convertino et al. (1990a) have also demonstrated a significant linear correlation between microgravity-induced changes in carotid-cardiac baroreflex gain and changes of systolic blood pressure during standing after 30 day bedrest. Baroreflex gain was reduced more in subjects who fainted during standing after bedrest than in those who did not (Convertino et al., 1990a).

Lower Body Negative Pressure. LBNP has been used extensively as a research tool to evaluate the cardiovascular response to orthostatic stress (Hoffler, 1977; Wolthuis et al., 1974). This technique has the inherent advantage of producing a gravity-independent redistribution of venous volume. Furthermore, because subjects remain supine during LBNP, measurement of physiologic responses is facilitated and

the liklihood of confounding activity in skeletal muscle is minimized (Convertino, 1987a).

Several investigators have demonstrated that LBNP at -40 to -50 mmHg results in blood pressure and heart rate changes similar to those seen with passive standing and head-up tilt (Hoffler, 1977; Musgrave et al., 1969; Wolthuis et al., 1974). Changes in maximal orthostatic capacity following bedrest or true microgravity exposure has also been determined with LBNP (Hordinsky et al., 1981; Sandler et al., 1985). Hoffler (1977) reported that 18 Apollo crewmembers exhibited elevations in heart rate that averaged 42% higher after flight during LBNP to -50 mmHg. Additionally, pulse pressure was reduced by 25% during the orthostatic stress with 7 astronauts experiencing pre-syncopal symptoms during their immediate post-flight LBNP test (Hoffler, 1977). Data from groundbased experiments suggest that prior to bedrest, 80-90% of all subjects can tolerate a minimum of 15 minutes of -50 mmHg without experiencing syncopal symptoms. Following bedrest, however, time to syncope is reduced by nearly 50% (Melada et al., 1975; Miller et al., 1964b; Sandler, 1980; Sandler and Winter, 1978; Stevens et al., 1966). Bedrested subjects exhibit heart rate responses to LBNP which are similar to those observed in flight crews following exposure to true microgravity with heart rate usually doubling during LBNP following weightlessness (Greenleaf et al., 1976, 1982). Interestingly, females

undergoing LBNP have shown greater increases in heart rate both before and after bedrest (Sandler and Winter, 1978).

2.6 Effects of Exercise on Baroreflex Function

2.6.1 Endurance Exercise Training

It is well-known that endurance (aerobic) exercise training of moderate intensity and long duration increases maximal oxygen uptake and blood volume (Saltin et al., 1968). Conversely, microgravity induces a significant reduction in both of these parameters (Convertino, 1987b; Fischer et al., 1967; Johnson et al., 1977a; Leach and Johnson, 1984). Interestingly, it has been reported that the incidence of orthostatic intolerance following exposure to actual spaceflight (Convertino, 1990; Hoffler, 1977) and simulated microgravity (Hyatt and West, 1977; Sandler, 1986) is closely related to the magnitude of blood volume loss. Therefore, it seems reasonable to consider that adherence to an endurance exercise training program while inhabiting a low gravity environment would maintain or increase blood volume and help defend against post-exposure loss of orthostatic tolerance. However, data from both spaceflight and ground-based experiments do not support this hypothesis.

Extensive endurance exercise training was used during the U.S. Skylab program and, although the conditioning program was effective in maintaining a high level of crew

physical fitness (Convertino, 1990), plasma volume was reduced and orthostatic instability was increased following return to earth (Convertino, 1990; Johnson et al., 1977b). Greenleaf et al. (1989) assessed the effects of exercise training during 30 days of bedrest on orthostatic hypotension. Cardiovascular responses and time to pre-syncope during 60° head-up tilt were measured in three groups of subjects who had performed no exercise, endurance exercise, or resistive exercise during the bedrest period. Only subjects who participated in the endurance exercise training program maintained plasma volume at pre-bedrest levels. However, post-bedrest tilt tolerance times were reduced by the same magnitude in all groups (Greenleaf et al., 1989). Since blood volume was maintained in the endurance-trained subjects, these data provide evidence that physiological mechanisms other than vascular volume must be important in determining orthostatic tolerance following prolonged exposure to microgravity.

In addition to reduced blood volume, baroreflex responses are impaired by microgravity and its analogs (Convertino et al., 1990a, 1991a, 1992; Eckberg and Fritsch, 1992; Fritsch et al., 1992; Nicogossian et al., 1991) with the degree of impairment related to the duration of exposure (Convertino et al., 1990a; White et al., 1991). Altered baroreflex function has also been associated with orthostatic instability following bedrest (Convertino et al., 1990a). The results from spaceflight (Johnson et al., 1977b) and

simulated microgravity (Greenleaf et al., 1989) studies suggest that training regimens designed to defend aerobic capacity and increase blood volume may not provide the appropriate stimuli to reverse or attenuate baroreflex impairment (Convertino et al., 1990b; Seals and Chase, 1989; Tatro et al., 1992).

2.6.2 Dynamic Maximal Exercise

There is evidence to suggest that graded, dynamic maximal exercise can acutely restore various cardiovascular and metabolic capacities attenuated by exposure to simulated microgravity or physical deconditioning. One bout of maximal exercise following 10 days of detraining returned insulin sensitivity to normal levels (Heath et al., 1983). Similarly, a single bout of exhaustive treadmill exercise at the end of 10 days of bedrest restored maximal oxygen uptake, heart rate, blood pressure, time to fatigue, and orthostatic stability to pre-bedrest levels within two hours of ambulation (Convertino, 1987b). Furthermore, performance of exhaustive exercise provided protection against orthostatic instability and syncope following water immersion (Stegemann et al., 1975).

There are also reports that a single bout of dynamic exercise enhances blood pressure regulation during the post-exercise recovery period. This observation has been attributed to an alteration in sensitivity of cardiovascular

reflexes mediated by arterial baroreceptors. Somers et al. (1985) reported a 50% increase in the gain of the cardiac baroreflex stimulus-response relationship 60 minutes after completion of fatiguing cycle exercise. Convertino and Adams (1991) observed a 92% increase in baroreflex sensitivity 3 hours after cessation of maximal leg exercise with the effect maintained for up to 24 hours. Also, a 32% increase in carotid baroreflex gain has been reported 24 hours after completion of a bout of resistance exercise in bedrested subjects (Convertino et al., 1992).

The mechanisms responsible for increased carotid baroreflex sensitivity following a single bout of intense exercise are unclear. Several investigators have reported rapid resetting of the baroreflex at the onset of exercise (Ebert, 1986; Hales and Ludbrook, 1988; Rowell, 1986; Shepherd and Mancia, 1986) due to either changes in central modulation of the reflex arc or to changes at the site of the mechanoreceptors (Chapleau and Abboud, 1989; Chapleau et al., 1989), but this seems an unlikely or incomplete explanation because resetting involves a shift of the stimulus-response curve along the stimulus axis without a change in sensitivity (gain) of the reflex. Similarly, alterations in ionic environment and circulating hormones, which have been shown to change arterial baroreceptor sensitivity (Holmes and Ledsome, 1984; Munch and Brown, 1985; Munch et al., 1987; Tomomatsu and Nishi, 1981), do not appear to explain changes in baroreflex sensitivity since elevations in these agents

caused by exercise are transient and return to baseline levels before changes in baroreflex function occur. Changes in vascular volume caused by acute exercise (Gillen et al., 1991) should have little contribution to the observed changes in baroreflex function since acute and chronic changes in blood volume are not related to alterations in the carotid-cardiac vagal baroreflex response (Convertino, 1992; Convertino et al., 1990a; Thompson et al., 1990). Input from muscle chemosensitive (metaboreceptor) afferents may also modulate autonomically-mediated blood pressure control mechanisms following exercise, possibly in the central cardiovascular centers of the brain stem (O'Leary, 1993), but this hypothesis awaits further research.

There is evidence to suggest that loading arterial baroreceptors by periodic elevation of systolic blood pressure may provide a stimulus to maintain or increase sensitivity of the baroreflexes. The observation of a reduced baroreflex gain following long-term restriction from a 1-g environment via spaceflight (Fritsch et al., 1992), bedrest (Convertino et al., 1990a, 1992; Eckberg and Fritsch, 1992), or chair confinement (Convertino et al., 1991) may underscore the importance of periodic loading and unloading of baroreceptors in maintaining reflex integrity. This hypothesis is supported by the observation that pulsatile increases in arterial pressure altered carotid baroreceptor sensitivity; an effect which persisted for several minutes after removal of the stimulus (Chapleau and Abboud, 1989).

Additional support comes from Eiken et al. (1992) who used a combination of submaximal exercise and lower body positive pressure to load the baroreceptors and observed an enhanced gain of the carotid-cardiac baroreflex.

The mechanisms by which pulsatile elevations of pressure induce changes in baroreflex sensitivity remain unclear. Chapleau and Abboud (1989) noted pulse pressure-induced baroreflex sensitization could not be explained by changes in viscoelastic properties of the carotid sinus nor by release of endothelial or ionic substances. Interestingly, increased pulse pressure appears to inhibit sympathetic nerve activity within the medullary cardiovascular centers possibly leading to central modulation of the baroreflex (Chapleau et al., 1989). Thus, increased pulse pressure which accompanies increased arterial pressure elicited by dynamic exercise may provide a stimulus which acts in the central nervous system to heighten the sensitivity of the carotid-cardiac baroreflex.

2.7 Summary

Reflex responses of the cardiovascular system to orthostasis are illustrated in Figure 2.1. With the onset of orthostatic stress, large quantities of blood accumulate in the lower extremities due to an increased head-to-toe hydrostatic gradient, thus compromising venous return, atrial filling,and cardiac output. Subsequent reductions of central

venous and arterial pressures elicit strong cardiopulmonary and arterial baroreceptor responses which initiate a cascade of neuro-humoral responses to restore blood pressure homeostasis. Failure of these mechanisms to respond with sufficient speed and magnitude will result in orthostatic intolerance and possible syncope.

Also indicated in Figure 2.1 are components of the blood pressure control system whose function is enhanced following performance of a single bout of maximal exercise. Specifically, it has been demonstrated that exhaustive exercise enhances carotid-cardiac baroreflex sensitivity and increases plasma volume, however, the importance of these adaptations to blood pressure stability during provocative orhtostatic testing has not been determined. Therefore, data presented in the following chapters provides evidence that exercise-induced increases of baroreflex sensitivity and plasma volume are associated with improved blood pressure control and increased orthostatic tolerance. These data also identify other blood pressure regulatory variables and their interactions influenced by exercise as well as identify areas for future research.

2.8 Conclusions

1. Arterial baroreceptors are mechano- (stretch) receptors which are stimulated by deformation of the vascular wall in which they are located. These receptors respond to

changes in intravascular pressure by initiating actions which return mean arterial pressure to homeostatic levels.

2. The bradycardic response to baroreceptor stimulation is mediated via vagal cholinergic mechanisms. Controversy remains as to mechanisms mediating the tachycardic response, although evidence exists which suggests both sympathetic and parasympathetic involvement.
3. Cardiopulmonary baroreceptors are located on the low pressure (venous) side of the circulation and repond to alterations in blood volume by eliciting reflex changes in systemic resistance.
4. Baroreflex function is evaluated via invasive and non-invasive techniques which test either integrated baroreceptor function or isolated reflex arcs. The method utilized to assess reflex function will determine the conclusions which can be drawn from the data.
5. The operating range of baroreflex stimulus-response relationships is labile and readily shifts in the direction of prevailing arterial pressure. There is evidence to suggest that increases in intravascular pressure which are pulsatile in nature prevent baroreflex resetting, but elicit changes in baroreceptor sensitivity. This may also be the case with activation of muscle chemosensitive receptors.

6. Circulating hormones such as norepinephrine, angiotensin II, vasopressin, and atrial natriuretic peptide modify baroreceptor activity.
7. Baroreflex function is sensitive to changes in the gravitational environment. This is readily demonstrated by observations of altered baroreflex function following prolonged spaceflight, bedrest, and chair confinement. Altered baroreflex activity may play a role in development of orthostatic hypotension following microgravity exposure.
8. Unlike conventional endurance exercise training which has no apparent effect on baroreceptor sensitivity, a single bout of maximal dynamic exercise appears to provide a stimulus which enhances sensitivity of the carotid-cardiac baroreflex and increases plasma volume. Although the mechanism of this action remains unclear, recent evidence in animal models suggests that pulsatile increases of arterial pressure may play a role. Also, afferent input from muscle chemosensitive receptors may influence sensitivity of baroreflexes, possibly through action in the medullary cardiovascular control centers.

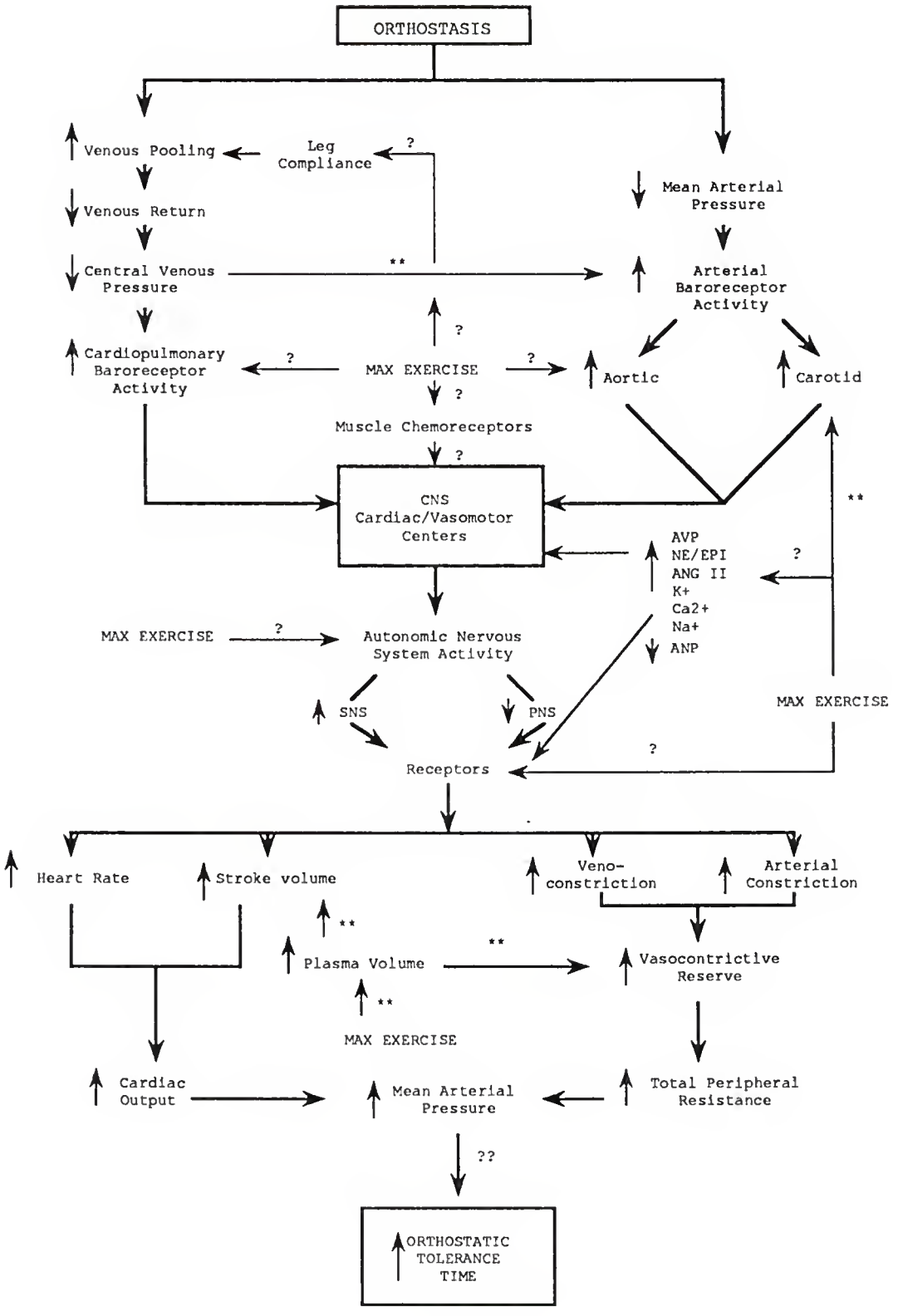

Figure 2.1. Cardiovascular reflex responses to orthostasis. Asterisks indicate where maximal exercise is known to impact mechanisms responsible for blood pressure control. Questions marks identify where exercise may potentially exert an effect, but confirmation awaits future research.

CHAPTER 3
GENERAL METHODOLOGY

3.1 Experimental Design

Both Studies 1 and 2 employed a cross-over design in which the order of experimental treatments was randomized. Using the subjects as their own controls minimized the potential for heterogeneous responses to experimental interventions across groups of different subjects and therefore served to decrease the probability of making a Type I error due to relatively small sample size.

3.2 Experimental Procedures and Protocols

3.2.1 Study 1

For Study 1, paraplegic subjects confined to wheelchairs due to complete traumatic spinal transection in the thoracic region were recruited. Because restriction to the seated posture has been associated with postural hypotension due to partial removal of the hydrostatic gradient along the longitudinal axis of the body (Blomqvist and Stone, 1983; Lamb et al., 1965), these subjects provided an accessible model of the long-term effects of microgravity on blood pressure homeostasis. All subjects completed two

experimental protocols. Each protocol involved measurement of heart rate (HR), blood pressure (BP), forearm vascular resistance (FVR), and vasoactive hormone (NE, AVP, PRA) responses before and during 15 minutes of 70° head-up tilt on two days: 1) 24 hours after acute intense arm exercise, and 2) during a control (no exercise) condition. Additionally, measurements of plasma volume, carotid-cardiac baroreflex relationship, leg vascular compliance, and hemodynamic responses to the Valsalva maneuver were made under the two experimental conditions. Test days were separated by one week and the two experimental conditions were given in random order. This study was conducted in the hospital laboratory at the Humana-Lucerne Spinal Injury Unit in Orlando, Florida.

3.2.2 Study 2

Study 2 required that ambulatory subjects be exposed to two 16 day periods of 6° head-down tilt (HDT) separated by 11 months. The experimental protocol for this study consisted of 4 days of ambulatory control (C1-C4) followed by 16 days of 6° HDT (BR1-BR16) and 2 days of post-HDT recovery (R1-R2) for a total of 22 days of confinement. During one HDT period, each subject performed an acute bout of graded exercise designed to elicit maximal effort twenty-four hours prior to re-ambulation (exercise condition). Following the other, subjects performed no exercise (control condition).

During both HDT periods, subjects lived 24 $h \cdot day^{-1}$ in the Human Research Facility at NASA-Ames Research Center, Moffett Field, CA and remained head-down without interruption for all daily activities except bathing, for which they were horizontal. The 16 day HDT period was chosen because it represents the maximum projected duration for Extended Duration Orbiter Space Shuttle missions. The 6° HDT posture was chosen because changes in cardiovascular reponses elicited by this posture are similar to those seen in actual spaceflight (Blomqvist and Stone, 1983; Convertino et al., 1989b). Subjects followed the same controlled diet (average daily caloric intake of 2500-2800 kcal: 45% carbohydrate, 38% fat, 17% protein) during both 22 day experimental periods. Dietary sodium and potassium were held constant at approximately 120 and 60-80 $mEq \cdot day^{-1}$ respectively, and fluid intake was *ad libitum*. The photoperiod was 16 hours light to 8 hours dark with lights on at 0700 hours. All measurements where conducted at the same time of day and in the same sequence before, during, and after HDT.

Plasma volume and the stimulus-response relationship of the carotid-cardiac baroreflex were measured on day C-4, day BR-16, and day R-1. A 30 ml antecubital venous blood sample was taken without stasis at 0700 h on these days for the baseline determination of plasma NE. On day C-4 and day R-1, each subject underwent a test of orthostatic tolerance using a graded LBNP protocol to pre-syncope. An additional blood sample was obtained immediately following termination of the

LBNP test to determine the catecholamine response to orthostasis.

3.3 Subjects

A total of 17 healthy, non-smoking, normotensive volunteers (15 males, 2 females; Table 3.1) gave their written consent to serve as subjects for these two investigations after they had been informed of all procedures and risks. All

Table 3.1. Physical characteristics of subjects.

	Study 1	Study 2
	n = 10	n = 7
Age, yr	36 ± 4	40 ± 2
Height, cm	185 ± 2	183 ± 2
Weight, kg	90 ± 7	81 ± 2

Values are mean ± SE.

procedures were approved by the Human Research Review Boards of NASA-Kennedy Space Center and NASA-Ames Research Center. Selection of subjects was based on results of a screening evaluation comprised of a detailed medical history, physical examination, blood chemistry analysis, urinalysis, chest X-ray, and resting and treadmill electrocardiogram. During an orientation session conducted prior to the study, all subjects were made familiar with the laboratory suroundings, laboratory personnel, and experimental procedures and

protocols. All subjects were asked to abstain from tobacco, alcohol, caffeine, medications, and conventional exercise for the duration of the studies.

3.4 Measurements and Techniques

3.4.1 Heart Rate and Blood Pressure

A direct writing, multi-pen recorder was used to transcribe the electrocardiogram (ECG) and BP recordings during all tests. Continuous HR was computed from the R-R interval of the ECG (Quinton Q4000 or Lifepack-6) from a three lead electrode placement. A Finapres fingercuff BP monitoring device (Ohmeda 2300) was used to provide continuous beat-by-beat measurement of peripheral arterial BP. The Finapres measures BP using a small finger cuff that contains a photoplethysmographic volume transducer and an inflatable air bladder. The cuff is connected to a fast-response servo control system that instantaneously regulates the pressure applied to the finger through the bladder and, thus the pressure applied to the walls of the arteries. As BP increases, the arterial wall expands, increasing the volume of the finger. This volume differential is measured by the plethysmographic transducer. The Finapres monitor responds to the increasing volume by increasing cuff pressure until the original arterial size and blood volume are again reached. The external pressure continuously adjusted by the

cuff closely follows the intra-arterial pressure within the finger, allowing measurement of the external pressure itself as a function of the arterial blood pressure (Boehmer, 1987). Thus, the Finapres provides a reliable non-invasive technique for the measurement of changes in beat-by-beat arterial blood pressures. Additionally, periodic BP measurements were made with a stethoscope and sphygmomanometer to verify the readings obtained from the Finapres.

3.4.2 Carotid Baroreflex Function

Carotid baroreceptor-cardiac reflex responses were measured with the method described by Sprenkle et al. (1986). Stimuli were delivered to the carotid baroreceptors by a computer-controlled motor-driven bellows which provided pressure steps to a silastic neck chamber covering the area of the carotid sinuses. An initial pressure of 40 mmHg was delivered to the chamber and maintained for four R waves. With the next R wave, the pressure sequentially stepped to approximately 25, 10, -5, -10, -20, -35, -50, and -65 mmHg followed by a return to ambient pressure. Pressure steps were triggered by R waves so that neck chamber pressures were superimposed upon naturally occurring cardiac pulses. To avoid respiration-related variations of cardiac vagal outflow, neck-pressure changes were applied only while subjects held their breath at mid-expiration. With this system, the pressure gradient across the arterial walls was

rapidly altered to produce reflex cardiac acceleration or slowing as measured by changes in the R-R interval. Carotid distending pressure (CDP) was calculated as systolic pressure minus neck-chamber pressure applied during each heart beat. This calculation assumes complete transfer of pressure in the neck chamber to the carotid arteries. A test session consisted of five successful applications of neck-pressure sequences. Each sequence lasted ~15 seconds and each test session lasted approximately 15 minutes. Individual trials were discarded immediately if the subject breathed during the stimulus sequence or if the neck chamber failed to seal adequately. Blood pressure was measured with a sphygmomanometer prior to the application of the stimulus.

From the average of the five trials, a stimulus-cardiac response curve was derived by plotting R-R intervals at each pressure step against their respective CDP. This plot was used to define threshold, saturation, and linear ranges of the sigmoid baroreceptor-cardiac reflex response. Previous studies have indicated that baroreceptor stimulus-sinus node response relationships measured in this manner are highly reproducible (Eckberg et al., 1992; Kasting et al., 1987). Sensitivity of the baroreflex was determined by calculating the maximum slope of this sigmoid curve. To determine the segment of steepest slope, least squares linear regression analysis was applied to every set of three consecutive points on the response relationship. According to Ludwig and

Convertino (1991), the maximum slope of the stimulus-response curve is the best indicator of baroreflex function.

R-R intervals were used to characterize the pressure input-neural output relationship because of the relationships that exist between R-R intervals and vagal-cardiac nerve activity and HR. R-R intervals are highly linear functions of vagal-cardiac nerve activity (Katona et al., 1970; Nicogossian et al., 1991). As a result of this linear relationship, it is possible to use changes of R-R intervals as surrogates for changes of vagal-cardiac nerve activity, and to compare responses before and after interventions such as HDT, which alter baseline R-R intervals. Because the relationship between HR and vagal-cardiac nerve activity is curvilinear, it is extraordinarily difficult to compare responses to baroreflex forcings when baseline HRs are different. Eckberg et al. (1976) have found that R-R interval responses to neck pressure sequences similar to those employed in this study are not reduced by beta-adrenergic blockade, but are nearly abolished by muscarinic blockade. Therefore, this measurement of carotid-cardiac baroreflex response focuses primarily on the vagal limb of the baroreceptor reflex.

3.4.3 Tests of Orthostatic Responses

Cardiovascular and vasoactive hormone responses to orthostasis were recorded in all subjects during either 70°

head-up tilt (Study 1) or lower body negative pressure (LBNP; Study 2). In either case, the duration of the orthostatic test was determined by any one or combination of the following criteria: (a) completion of a pre-determined time limit; (b) onset of presyncopal symptoms such as grey-out, a precipitous fall in systolic blood pressure greater than 15 mmHg, and/or a sudden bradycardia greater than 15 bpm between adjacent 1-min measurements; (c) progressive diminution of SBP below 80 mmHg; and (d) voluntary subject termination due to discomfort such as sweating, nausea, or dizziness (Sather et al., 1986).

70° Head-Up Tilt. A motorized tilt table elevated subjects from the supine posture to the 70° head-up position within 10 seconds. Subjects remained in this position for 15 minutes or until the onset of pre-syncopal symptoms. During tilt, beat-to-beat HR was continually recorded. Blood pressure was measured on the right arm at 2 minute intervals while forearm blood flow was measured on the left arm according to the protocol described below. At the conclusion of the test, the subject was quickly returned to the supine posture and a blood sample was drawn within 30 seconds to be analyzed for NE, AVP, and PRA. An identical procedure was repeated one week later to measure the same variables under the remaining treatment condition.

Lower Body Negative Pressure. LBNP is a means of producing circulatory alterations similar to those encountered when changing posture from supine to upright

standing. Following instrumentation for measurement of HR, BP, and forearm bloodflow, subjects in Study 2 were assisted into the supine position within a wooden LBNP pressure chamber. A foam-padded saddle was adjusted to comfortably support and stabilize the subject in the chamber. The location of the saddle was noted to ensure consistency in subject positioning during all LBNP tests. A rubberized skirt was used to secure an airtight seal between the chamber and the waist (identified as the iliac crests) of the subject. Orthostatic tolerance was determined by progressively reducing the pressure around the lower body relative to ambient pressure. The protocol consisted of a 5 minute resting period followed by decompression to -5, -10, -15, -30, and -40 mmHg for 2, 2, 15, 15, and 5 minutes, respectively. Supplemental 10-mmHg reductions in pressure every 2 minutes were added until test termination. Immediately following cessation of the test, an antecubital blood sample was obtained for determination of NE.

3.4.4 Blood and Plasma Volume

Plasma volume was determined using a modified Evans blue dye (T-1828; Macarthy Medical, Romford, Essex, UK) dilution method (Greenleaf et al., 1979). After a minimum of 30 minutes supine rest, a control baseline sample was drawn followed by an intravenous injection of 11.5 mg of dye pre-diluted with isotonic saline (2.5 ml) through a sterile 0.45

μm filter. A 5-ml aliquot of the control baseline sample was used to determine the pre-injection dye concentration. Plasma (1 ml) from a 10-minute post-injection blood sample was passed through a wood-cellulose powder (Solka-Floc SW-40A) chromatographic column so that the dye could be absorbed after it had been separated from the albumin by the action of a detergent (Teepol 610 in 2% Na_2HPO_4). In addition, interfering substances such as pigments, proteins, and chylomicrons were washed from the column with 2% Na_2HPO_4. The absorbed dye was eluted from the column using a 1:1 water-acetone solution (pH 7.0) and collected in a 10-ml volumetric flask. The post-injection solution was compared with 1-ml samples from a pre-injection time (zero control) and a standard dye solution (1:50 dilution with distilled water). All samples were read at 615 nm with a spectrophotometer. Plasma volume was calculated using a standard dilution equation as follows:

$$PV = [(V*D)*(St*v)]/(1.03*T)$$

Where

PV	=	plasma volume (ml)
V	=	volume of dye injected (ml)
D	=	dilution of standard (1:250)
St	=	absorbance of standard
v	=	volume of sample extracted (ml)
T	=	absorbance of plasma sample
1.03	=	correction factor for dye uptake by tissues

Hemoglobin concentration was determined with triplicate measurements using using the Coulter S+4 system. Hematocrit (Hct) was measured in triplicate with a microhematocrit centrifuge and a micro-capillary tube reader. Total blood

volume (TBV) was calculated from the plasma volume and Hct using the following equation (Convertino et al., 1980):

$$TBV\ (ml) = PV/(1.0 - 0.91\ Hct)$$

Where

0.91 = correction factor for venous/total body hematocrit (Chaplin et al, 1953)

Greenleaf et al. (1979) have reported that, using these procedures, the test-retest correlation coefficient for blood volume was 0.969 (N = 12) and the day-to-day variation was 82 ml (1.5%, N = 17) over 4 days, 75 ml (1.5%, N = 19) over 8 days, and 56 ml (1.1%, N = 23) over 15 days.

3.4.5 Vasoactive Hormones

Antecubital venous blood samples (30 ml) were drawn without stasis for the determination of NE, AVP, and PRA. Immediately following each withdrawal, 21 ml of whole blood was taken from the vacutainer and transferred to a chilled tube containing sodium EDTA. The remaining 9 ml was introduced into a lithium heparinized tube. Whole blood was also taken directly from the EDTA tube for triplicate microhematocrit (approximately 0.5 ml) and for hemoglobin (approximateley 0.5 ml) measures. The remaining EDTA-treated whole blood was centrifuged at 2000 g for 15 min at 4°C. Immediately after centrifugation, the plasma was aliquoted for NE, AVP, and PRA and stored at -60°C until hormonal assays were performed.

Norepinephrine. Plasma NE concentrations were measured by high performance liquid chromatography (Waters). NE was extracted by absorbing plasma samples onto alumina. Following washing of the absorbed alumina with a dilute buffer solution, catecholamines were eluted from the alumina when treated with an acidic solution. 3,4-Dihydroxybenzylamine was used as an internal standard and extraction efficiency of NE was based on the extraction of known standards. After extraction, the samples were assayed using a Waters 712 Wisp to inject the samples onto a reverse phase C^{18} column. A Waters 460 electrochemical detector was used to determine the concentrations of NE in the samples. The auto-injector and detector were interfaced with a Digital 380 computer using Waters software. The within assay coefficient of variability was 1.4% and between assay coefficient of variability was 3.8%.

Arginine Vasopressin. Radio-immunoassay (RIA) procedures were used to analyze plasma for AVP (Instar Nuclear Corp.). To determine AVP, samples were extracted using ODS-silica columns and then assayed using a disequilibrium RIA procedure. The sample and first antibody were incubated for 18 h at 2-8°C. ^{125}I tracer was added followed by a second incubation at 2-8°C. A second antibody precipitating complex was added for phase separation. The supernatant was then poured off and the precipitate counted in a gamma counter. Spiked recovery was 92%, sensitivity was

0.5 $pg \cdot ml^{-1}$, within assay coefficient of variability was 2.8% and between assay coefficient of variability was 9.9%.

Plasma Renin Activity. To determine PRA, an EDTA plasma sample was adjusted to pH 6 with maleic acid. Half of the sample was incubated in a 37°C water bath for 2 h and the other half was kept in an ice bath at 4°C. The incubated portion reflects the circulating level plus the quantity of ANG I generated through the action of the renin in the plasma sample. The net quantity of angiotensin I at 37°C was calculated by subtracting the ANG I level in the 4°C sample from the ANG I level in the 37°C sample. Measurement of PRA was performed by a RIA competitive binding procedure using a specific antibody, a radiolabeled antigen, a pure sample of antigen used as a reference standard, and a separation medium. The amount of unlabeled antigen in the sample being analyzed was determined by comparing the assay results to a standard curve prepared with known amounts of the unlabeled antigen. Recovery efficiency was 96%, sensitivity was 0.1 $ng \cdot ml \cdot^{-1} h^{-1}$, within assay coefficient of variability was 2.7% and between assay coefficient of variability was 5.5%.

3.4.6 Forearm Blood Flow

Forearm blood flow (FBF) was measured using venous occlusion plethymography employing a dual loop mercury-in-silastic strain gauge (Whitney, 1953). The strain gauge was calibrated before and after all experimental sessions in

which FBF was measured and the ouput of the strain gauge circuitry was directed to a 2-channel chart recorder (Gould). Approximately one minute prior to each blood flow measurement, a wrist occlusion cuff was inflated to +250 mmHg to impede blood flow to the hand. This cuff remained inflated for the duration of blood flow determination. Following wrist cuff inflation, a cuff placed around the arm just above the elbow was inflated to 40 mmHg at 10 second intervals for two minutes to prevent the efflux of venous blood from the forearm, thus causing the strain gauge to stretch. By treating the forearm as a cyclinder in which an increase in volume results in an increase in cylinder circumference but not length, the amount of stretching can then be used as a quantitative index of venous volume. Blood flow was calculated by manually drawing a tangent to the line of the first three pulse waves and measuring this slope in terms of percent circumference change vs. time. Percent volume change per minute ($ml \cdot 100ml^{-1} \cdot min^{-1}$) was calculated from circumference change. Therefore, the linear change in forearm circumference during the venous occlusion was used to calculate blood flow as follows:

$$V = [(2 * C_2) * C_1^{-1}] * 100$$

$$FBF = V * T^{-1}$$

Where:

C_1 = initial forearm circumference (cm)
C_2 = change in forearm circumference (cm)
V = change in forearm volume ($ml \cdot 100$ ml tissue^{-1})
T = time for measurement (min)

Forearm blood flow measurements were averaged to provide one value representing mean forearm blood flow for a given perturbation. Forearm vascular resistance (FVR) was calculated by dividing the mean arterial pressure by forearm blood flow.

3.4.7 Leg Compliance

Compliance of the left leg was measured during supine rest using a Whitney strain gauge placed at the point of greatest calf circumference (Thornton and Hoffler, 1977). Following 30 minutes of supine control, the left leg was slightly elevated (~4 inches) at the ankle and an occlusion cuff placed just above the knee was inflated to 40 mmHg for 120 seconds. Leg compliance was calculated by dividing the volume change ($ml \cdot 100ml^{-1}$) at a plateau (i.e., point at which venous pressure equals cuff pressure) by the cuff pressure and expressed as $\Delta vol\% \cdot \Delta mmHg^{-1}$. The value for leg compliance was multiplied by 100 for convenience.

3.4.8 Leg Volume

Changes in left leg volume during each LBNP stage were measured with a strain gauge placed around the point of maximal calf girth. Percent changes in calf volume ($ml \cdot 100\ ml^{-1}$) were calculated from circumference changes. For small changes in gauge circumference, resultant cross-sectional area (and volume) changes were doubled.

3.4.9 Exercise Bout

An acute bout of exercise was performed by all subjects 24 hours prior to measurement of autonomic, hormone, cardiac, and hemodynamic activity during the orthostatic tests. For Study 1, subjects performed a multistage graded exercise protocol utilizing a Monark arm-crank ergometer. After a 4-minute warm-up against no load, the work rate was set at 10 watts increased by 5 watts every 2-minutes until fatigue. For Study 2, exercise consisted of graded work intensities to volitional fatigue utilizing a Quinton (model 845B) electronically-braked supine cycle ergometer. After a 4-minute warm-up at 200 $kpm \cdot min^{-1}$, the work rate was increased by 100 $kpm \cdot min^{-1}$ every minute until exhaustion. During both tests, a tachometer placed at eye level assisted the subject in maintaining a rotational cadence of 60-70 rpm. Both arm-crank and leg exercise protocols were designed so that the duration of the treatment was approximatley 15 minutes. Continuous, multistage graded workrate protocols designed to elicit maximal effort were chosen since similar tests have provided evidence of causing acute elevations in baroreflex sensitivity in normotensive subjects (Convertino and Adams, 1991) and hypertensive patients (Somers et al., 1985). HR was recorded during the last 15 seconds of each minute while BP was measured by brachial artery auscultation before and immediately after cessation of exercise. Subjects were

verbally encouraged to achieve a maximal effort. The exercise bout was terminated when the subject reached volitional fatigue and was unable to maintain the required cadence for a period exceeding 15 seconds.

3.4.10 Valsalva Maneuver

Subjects in both studies performed three Valsalva maneuvers according to a strict protocol at a controlled expiratory pressure of 30 mmHg. Each trial included a 30-second baseline period of quiet breathing, a 15-second strain period, and a 2-minute post-strain period. After instruction in the technique and several practice trials, subjects were asked to take a normal inhalation and blow into a mouthpiece connected to a pressure transducer. A small leak in the system prevented the subject from maintaining the expiratory pressure by occluding the glottis. A pressure gauge positioned in front of the subject provided feedback on the expiratory pressure. The expiratory pressure was used as an index of intra-thoracic pressure changes during Valsalva maneuvers (Elisberg, 1963). Continuous HR was recorded with an ECG and beat-to-beat BP responses were measured with the Finapres. Excellent estimates of directly measured intra-arterial pressures during Valsalva maneuvers have been demonstrated with this device (Imholz et al., 1988). Both HR and BP responses were saved as a digital record and data from the three trials were averaged.

HR and BP changes during the four phases of the Valsalva maneuver (Hamilton et al., 1936) were analyzed in a phase-by-phase manner (Ten Harkel et al., 1990). For comparison between experimental conditions, blood pressure responses (ΔMAP) to each phase were quantified as follows (see Fig. 3.1): phase I) peak MAP during early straining - baseline MAP (point b - point a); early phase II) peak phase I MAP - lowest mid-strain MAP (point c - point b); late phase II) peak late strain MAP - baseline MAP (point d - point a); phase III) peak phase II MAP - MAP immediately followingtermination of strain (point d - point e); and phase IV) peak post-strain MAP - phase III MAP (point f - point e). Changes in heart rate (ΔHR) were also determined during all four phases. Change in HR to change in MAP (ΔHR/ΔMAP) during phases II and IV was used as an index of cardiac baroreflex sensitivity because of its usefulness in describing integrated baroreflex function (Korner et al. 1976, Porth et al., 1984; Smith et al., 1987b). Integrated baroreflex-mediated vasoconstriction was assessed by measuring the late phase II rise in MAP (Korner et al., 1976; Sandroni et al., 1991).

3.4.11 Statistical Analysis

For Study 1, standard descriptive statistics were performed on each of the response variables of interest with results presented as means ± SE. The average HR and BP to

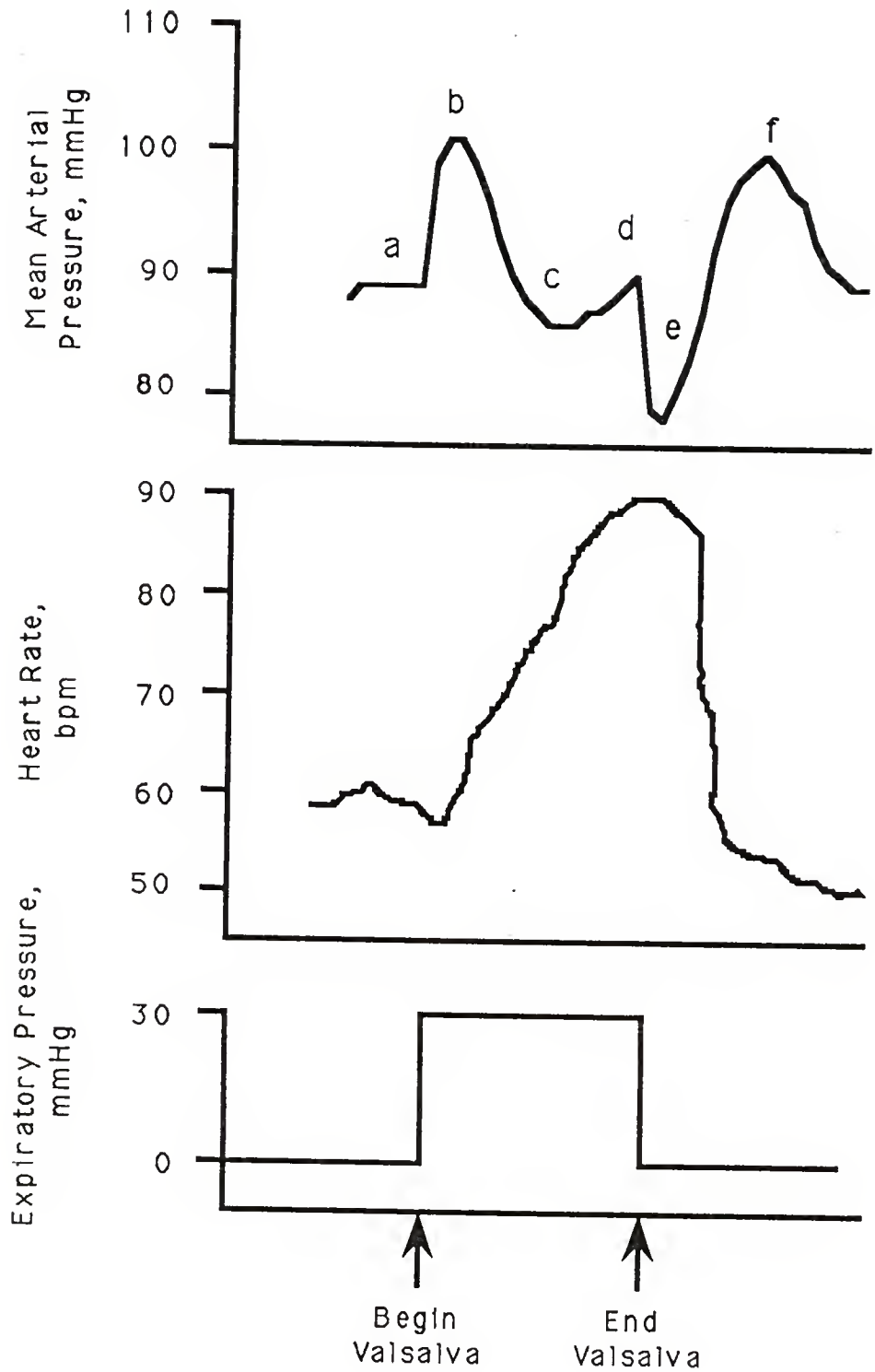

Figure 3.1. Representative mean arterial pressure and heart rate reponses to a 15-s Valsalva maneuver at an expiratory pressure of 30 mmHg. Also indicated are characteristic sample points used for analysis (see text).

head-up tilt as well as baseline plasma volume, leg compliance, Valsalva maneuver, and carotid-cardiac baroreflex gain under the two treatment conditions were compared using paired-difference *t* statistics. Vasoactive hormone and forearm vascular resistance responses were compared with a repeated measures two-way ANOVA.

For Study 2, standard descriptive statistics were also calculated for baseline levels of HR, BP, slope of the carotid-cardiac baroreflex, vascular resistance, leg volume, plasma volume, and NE with results presented as means ± SE. Since each subject received all levels of both factors (subjects were crossed with treatments) and tests were administered in a set sequence over time, the subjects acted as blocks and multivariate profile analyses were used to analyze these data. This approach to the analysis of repeated measures yields exact tests of sources of variation associated with treatment conditions including the treatment condition by HDT interaction (i.e., the test for parallel profiles). Statistical probabilities associated with the main effect of HDT, LBNP, and the HDT by LBNP interaction reflect the chances of observing a difference as large or larger than the one observed if in fact the actual difference was zero.

CHAPTER 4
AUTONOMIC FUNCTIONS AND ORTHOSTATIC RESPONSES 24 HOURS AFTER ACUTE INTENSE EXERCISE IN PARAPLEGIC SUBJECTS[1]

4.1 Abstract

We tested the hypothesis that a bout of graded arm exercise designed to elicit maximal effort would increase the sensitivity of autonomically-mediated baroreflexes and enhance blood pressure stability during 70° HUT in subjects prone to postural hypotension. Therefore, we measured HR, BP, FVR, and vasoactive hormone responses before and during 15 min of 70° HUT in 10 paraplegic subjects (21-65 yr) on two occasions: 1) 24 h after maximal arm-crank exercise (post-exercise) and 2) without exercise (control). During HUT, HR increased 30 bpm in both post-exercise and control, but the reduction in SBP during control (-12.0 ± 4.6 mmHg) was larger (P= 0.017) than that during HUT following exercise (-0.3 ± 4.3 mmHg). The post-exercise increase in FVR from supine to HUT of 17.0 ± 2.4 to 24.8 ± 3.2 PRU was greater (P= 0.042) than the increase observed during control (18.3 ± 3.7 to 19.5 ± 3.1 PRU). The gain of the carotid-cardiac baroreflex was also increased (P = 0.049) following exercise. Responses in norepinephrine, vasopressin, and plasma renin-angiotensin

[1]This work was supported in part by a NASA grant administered under contract NAS10-11624.

induced by HUT were similar for control and post-exercise and there was no difference in either leg compliance or plasma volume between the two conditions. Additionally, HR and SBP responses to phases II and IV of the Valsalva maneuver, indices of integrated baroreflex sensitivity, were enhanced ($P < 0.05$) following maximal exercise compared to control. Thus, acute intense exercise eliminated orthostatic hypotension in paraplegics and was associated with increased FVR and baroreflex sensitivity and was independent of blood volume changes.

4.2 Introduction

Cardiovascular reflexes mediated by the baroreceptors regulate cardiac output and systemic peripheral resistance to maintain mean arterial pressure during an orthostatic challenge. Since blood pressure varies as the product of heart rate, stroke volume, and vascular resistance, failure of these fast-acting autonomic reflexes to counteract orthostatically-induced reductions in cardiac output can lead to hypotension and possible syncope. A disruption in the integrity of these reflexes has been associated with compromised blood pressure control following prolonged bedrest (Convertino et al., 1990a) and chair confinement (Convertino et al., 1991) as indicated by an attenuation of orthostatic tachycardia and a reduction in the gain of the carotid-cardiac baroreflex (Convertino et al., 1990a, 1991).

A single bout of dynamic exercise has been reported to increase the sensitivity of cardiovascular reflexes and enhance the maintenance of blood pressure during the post-exercise recovery period. Acute graded exercise to exhaustion increased carotid baroreceptor stimulus-cardiac reflex response by 60 min post-exercise (Somers et al., 1986) and lasted as long as 24 h after exercise (Convertino and Adams, 1991; Convertino et al., 1992). A single bout of intense treadmill exercise at the end of 10 days of bedrest restored heart rate, blood pressure, and orthostatic stability to pre-bedrest levels within 2 h of ambulation (Convertino, 1987b) and reversed fainting episodes after 6 h of water immersion (Stegemann et al., 1975). These observations suggest that exercise of this type can enhance the function of cardiovascular reflexes to maintain blood pressure during orthostasis and may provide effective countermeasure treatment for orthostatic hypotension. Therefore, we tested the hypothesis that a bout of maximal arm exercise would increase the sensitivity of autonomically-mediated baroreflexes and enhance blood pressure stability during an orthostatic challenge. Paraplegic subjects were chosen because postural hypotension has been associated with prolonged wheelchair confinement (Convertino et al., 1991). We recently reported stable blood pressure during head-up tilt 24 h after maximal exercise was associated with an increased sensitivity of the carotid-cardiac baroreflex (Engelke et al., 1992). However, increased gain of this

vagally-mediated cardiac reflex response could not fully explain the elimination of orthostatic hypotension observed in the post-exercise condition. Consequently, we now report integrated arterial and cardiopulmonary baroreflex responses as well as heart rate, blood pressure, forearm vascular resistance, leg compliance, and vasoactive hormones during 70° HUT in 10 paraplegic subjects 24 h after a bout of dynamic arm exercise.

4.3 Methods

Eight male and two female sedentary paraplegics subjects [mean (±SE) age of 36 ± 4 yr, height of 185 ± 2 cm, and weight of 90 ± 7 kg] who had experienced traumatic spinal cord transection in the thoracic region (range of injury: T1-T12) gave their written consent to serve as subjects for this investigation after they had been informed of all procedures and risks. Subjects had been confined to their wheelchairs for an average of 118 ± 21 mo and none had attained the upright posture for at least 6 mo prior to the beginning of the study. Subjects completed two experimental protocols which involved measurement of HR, systolic and diastolic BP, FVR, and vasoactive hormone responses before and during 15 min of 70° HUT. Additionally, measurements of plasma volume, carotid-cardiac baroreflex relationship, leg compliance, and responses to the Valsalva maneuver were made.

Upon arrival at the laboratory, the subject assumed the supine posture on a tilt table. After 30 min quiet rest, a blood sample was obtained to determine plasma volume and pre-tilt plasma levels of NE, AVP, and PRA. Following the blood sample, a test was conducted for measurement of the carotid baroreceptor stimulus-cardiac response relationship. Leg compliance, and HR and BP responses to the Valsalva maneuver were also determined at this time. Following these measurements, each subject underwent an orthostatic test on the tilt table. Subjects remained in the head-up position for 15 min or until the onset of pre-syncopal symptoms. At the conclusion of the tilt, the subject was quickly returned to the supine posture and a blood sample was drawn within 30 s to be analyzed for NE, AVP, and PRA.

4.4 Results

Exercise bouts. The final work rate at volitional fatigue averaged 38 ± 1 watts and was attained after a mean time of 14 ± 1 min. HR, systolic and diastolic BP at termination averaged 175 ± 2 bpm, 165 ± 2 mmHg, and 71 ± 1 mmHg, respectively.

Response to 70° HUT. Supine hemodynamics and vasoactive hormones were not different between treatments (Table 4.1). During HUT, HR was elevated ($P = 0.001$) by 29 bpm and 30 bpm under control and exercise conditions, respectively. However, during HUT in the control condition, SBP decreased

(P = 0.025) from 118 ± 5 to 106 ± 9 mmHg, while there was essentially no change in SBP during HUT 24 h after maximal exercise (116 ± 5 to 113 ± 5 mmHg). Average reduction in SBP during control HUT (-12.0 ± 4.6 mmHg) was larger (P = 0.017) than that during HUT following exercise (-3.1 ± 3.9 mmHg). DBP during HUT was not altered in either condition.

Table 4.1. Baseline hemodynamic, vasoactive hormone, plasma volume, and vascular resistance values during control and 24 hours after exhaustive exercise.

	Control	Exercise	P value
HR, bpm	61 ± 4	60 ± 6	0.427
SBP, mmHg	118 ± 5	116 ± 4	0.811
DBP, mmHg	77 ± 4	76 ± 4	0.568
NE, $pg \cdot ml^{-1}$	322 ± 53	304 ± 57	0.562
AVP, $pg \cdot ml^{-1}$	1.9 ± 0.1	1.9 ± 0.1	0.933
PRA, $pg \cdot ml^{-1}$	0.77 ± 0.3	0.77 ± 0.2	0.775
PV, ml	3020 ± 132	3191 ± 210	0.459
FVR, pru	18 ± 4	17 ± 2	0.697

Values are mean ± SE.

Leg Compliance. Left calf compliance in the post-exercise condition was not different (P = 0.438) than that recorded during control period (1.8 ± 0.2 vs. 2.1 ± 0.4 $ml \cdot mmHg^{-1}$).

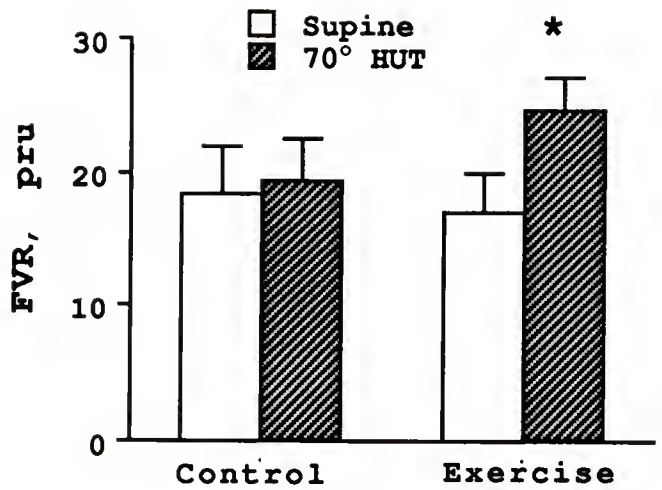

Figure 4.1. Comparison of forearm vascular resistance (FVR) in the supine posture (open bars) and after 15 min of 70° head-up tilt (HUT; closed bars) in the control and exercise conditions. Asterisks indicate $P < 0.05$ vs. corresponding values. Bars with 'T' lines represent means ± SE.

Baroreflex Responses. As illustrated in Figure 4.1, maximal exercise elicited greater ($P = 0.042$) FVR during HUT compared to the control condition. Pre-tilt baseline FVR was similar between control and exercise conditions, and was highly correlated ($r= 0.84$; $P= 0.03$). Mean HR and BP responses to the Valsalva strain in the control and post-exercise condition are illustrated in Figures 4.2A and 4.2B, respectively. Comparisons between HR and SBP changes during the four Valsalva maneuver phases are presented in Table 4.2. During late phase II, ΔSBP was four-fold greater ($P = 0.002$) 24 h post-exercise than ΔSBP measured at a similar time in the non-exercise condition. Also, the ΔHR/ΔSBP during phase IV was 40% greater ($P = 0.023$) in the post-exercise condition

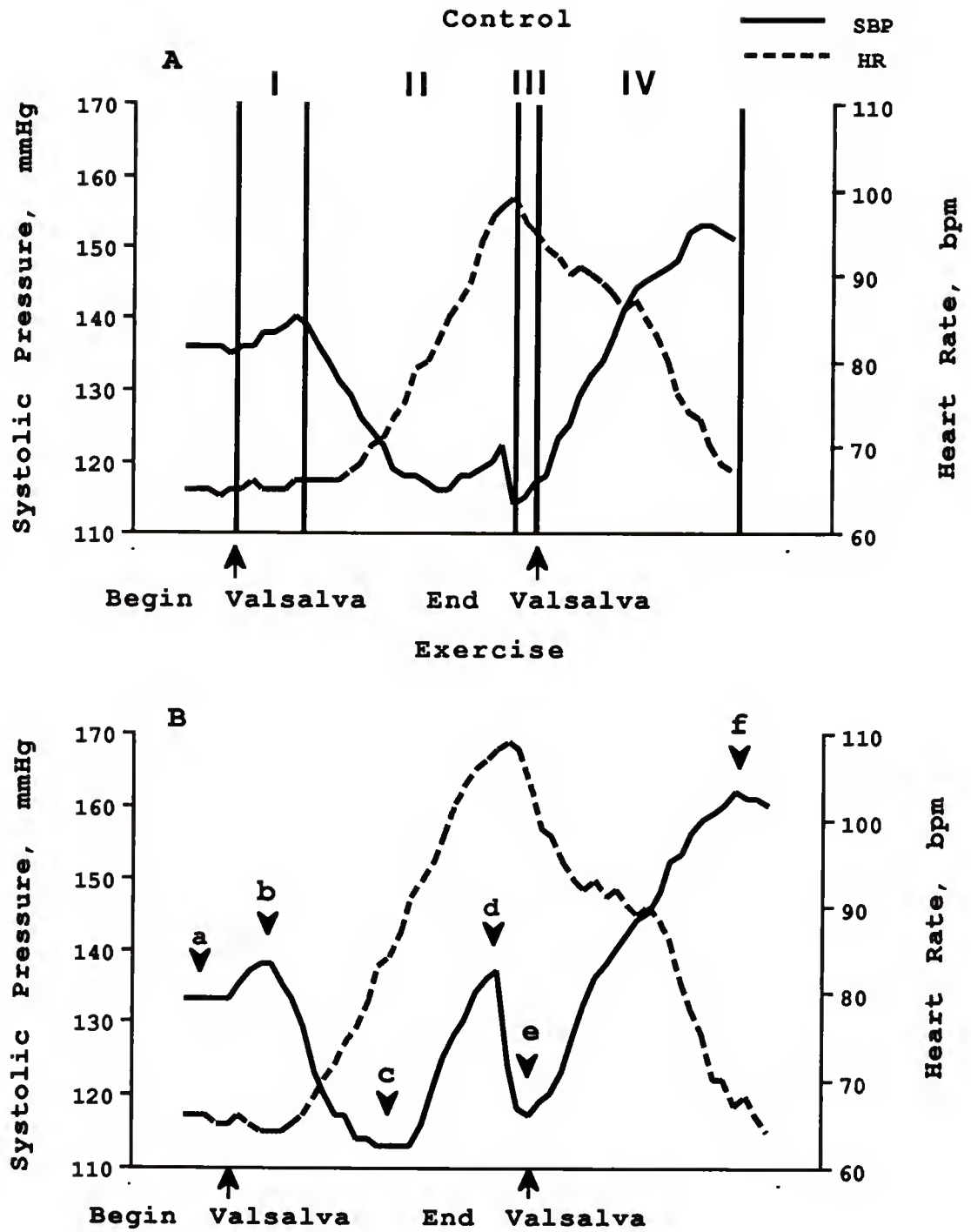

Figure 4.2. Mean heart rate (broken line) and systolic blood pressure (solid line) responses to the Valsalava maneuver in the control (panel A) and 24 h post-exercise (panel B) conditions. Panel A also indicates the four phases of the maneuver while panel B illustrates the characteristic sample points used for analysis (see text).

Table 4.2. Mean hemodynamic responses to the Valsalva maneuver.

	Control	Exercise	P value
Baseline HR, bpm	66 ± 2	63 ± 3	0.252
Baseline SBP, mmHg	137 ± 4	134 ± 4	0.416
Phase I			
ΔHR, bpm	-1 ± .4	-1 ± .3	0.477
ΔSBP, mmHg	3 ± .6	4 ± .7	0.338
ΔHR/ΔSBP, b·mmHg^{-1}	.33 ± .1	.25 ± .1	0.385
Phase II			
ΔHR, bpm	33 ± 3	43 ± 2	0.003
early ΔSBP, mmHg	-23 ± 3	-24 ± 3	0.576
ΔHR/ΔSBP, b·mmHg^{-1}	1.4 ± .3	1.7 ± .3	0.559
late ΔSBP, mmHg	-15 ± 4	4 ± 5	0.002
Phase IV			
ΔHR, bpm	-31 ± 6	-44 ± 6	0.005
ΔSBP, mmHg	44 ± 7	47 ± 6	0.739
ΔHR/ΔSBP, b·mmHg^{-1}	.7 ± .1	1.0 ± .1	0.023

Values are mean ± SE.

(1.01 ± .14) than that during the control period (.72 ± .11). There was no distinction between groups in ΔHR/ΔSBP during phase I. Phase III was brief and characterized by reductions in SBP and HR brought about by a decrease in mechanical compression of the thoracic cavity occurring as a result of the termination of the strain.

Mean carotid baroreflex stimulus-response relationships for all subjects during control and after exercise are illustrated in Figure 4.3. Mean maximum slope of the carotid-cardiac baroreflex reponses was increased (P = 0.049) 24 h following intense exercise (6.2 ± 1.7 msec·mmHg^{-1}) compared to the control condition (3.3 ± 0.6 msec·mmHg^{-1}).

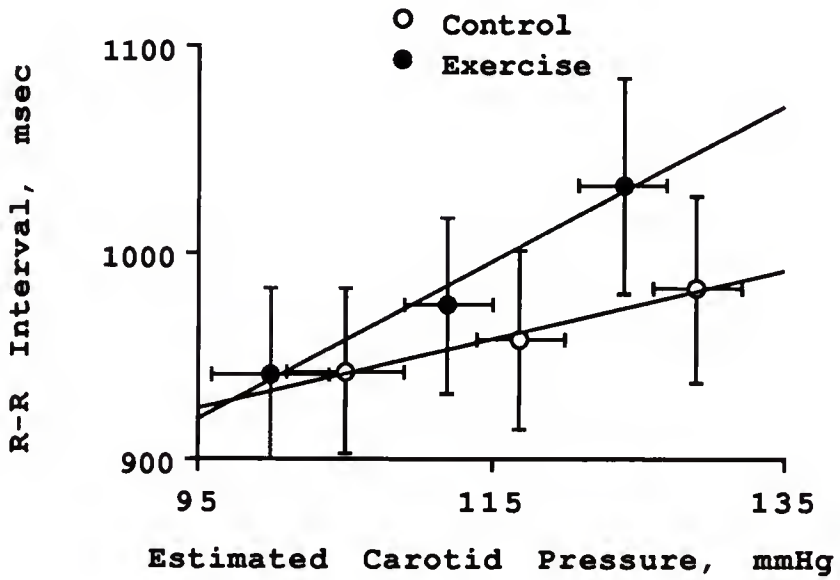

Figure 4.3. Carotid-cardiac baroreflex stimulus-response relationships, plotted over the range of pressures from which maximum slopes were derived, during control (open circles) and 24 h after exercise (closed circles). Bars with 'T' lines represent means ± SE.

Circulating Volume and Hormone Responses. NE, AVP, and PRA increased from supine to HUT (P < 0.05), but were similar for control and post-exercise (Fig. 4.4). Plasma volume measured 24 h after acute exercise (3020 ± 132 ml) was

indistinguishable (P = 0.459) from the volume measured on the control day (3191 ± 210 ml).

4.5 Discussion

To test the hypothesis that acute exercise designed to elicit maximal effort can sensitize autonomically-mediated cardiovascular reflex responses and ameliorate hypotension during an orthostatic challenge, we measured cardiac baroreflex responses and changes in HR, BP, and FVR to 15 min of 70° HUT in paraplegic subjects 24 h after intense arm-crank exercise. We also measured the plasma concentration of vasoactive hormones immediately following HUT. The primary finding of this study was that 24 h after exercise, the elimination of orthostatic hypotension during 70° HUT was associated with increased baroreflex control of heart rate and FVR, and independent of changes in blood volume and leg compliance compared to the control condition. Further, the observed improvement in systemic resistance occurred without alterations in circulating NE, AVP, and PRA.

A 10% expansion in plasma volume was induced 24 h after the performance of maximal leg exercise in ambulatory subjects (Gillen et al., 1992). If hypervolemia occurred in our subjects, it is possible that the improved blood pressure maintenance 24 h after exercise was associated with enhanced cardiac filling (Frank-Starling effect). Against expectations

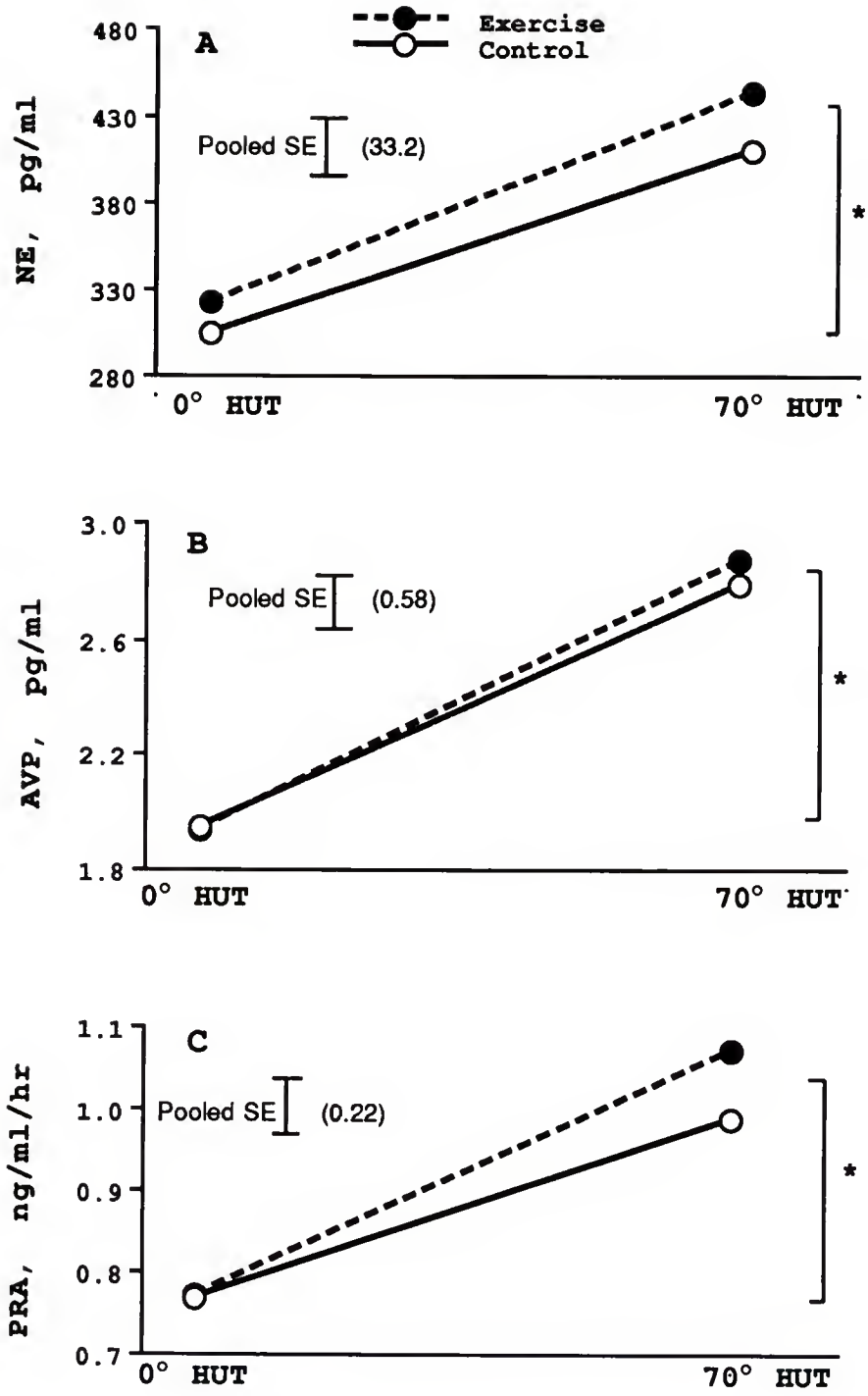

Figure 4.4. Circulating hormone levels during supine (0°) baseline and at the end of 70° HUT during control (open circles, solid lines) and post-exercise conditions (closed circles, broken lines). Asterisks indicate $P < 0.05$ compared with corresponding supine values.

plasma volume measured 24 h post-exercise in our subjects was not different than in the control condition. The lack of a hypervolemic effect is unclear, but may be associated with the lesser muscle mass and time to exhaustion involved in arm-cranking compared to leg exercise. In any event, improved blood pressure stability during HUT 24 h post-exercise could not be explained by blood volume changes.

Cardiac and hemodynamic responses during orthostatism may be influenced by leg venous compliance. An inverse relationship between leg compliance and tolerance to lower body negative pressure has been reported (Luft et al., 1976), suggesting that the capacity to maintain venous return, cardiac output and systemic arterial pressure during an orthostatic challenge is dependent on the amount of blood pooled in the lower extremities. As expected with the abolishment of sympathetic outflow to leg veins and arterioles as well as muscle tone in these patients, leg compliance in the present study was similar between control and exercise conditions. This finding eliminated the possibility that maintained blood pressure during HUT following exercise could be explained by differences in leg compliance. Unexpectedly, mean leg compliance measured in our paraplegic subjects (1.8 $ml \cdot mmHg^{-1}$) is approximately 30% to 60% less than values (2.6 - 4.4 $ml \cdot mmHg^{-1}$) reported for able-bodied subjects (Convertino et al., 1988, 1989b). These comparisons raise the possibility that marked reductions in

venous compliance in wheelchair-confined subjects may provide some protection against orthostatic challenge.

Since circulating vasoactive hormones, blood volume, and leg compliance were unaltered by exercise, increased sensitivity of reflexes mediated by baroreceptors probably represented the most contributing mechanism to the differences in maintenance of arterial pressure during HUT 24 h after exercise. Among these reflexes, it has been demonstrated that the sensitivity of the carotid-cardiac baroreflex can be increased for as long as 24 h following completion of a single bout of maximal exercise (Convertino and Adams, 1991). Our present data not only confirm this finding but also provide evidence to suggest that baroreflex responses associated with control of vascular resistance are increased by exercise as well. SBP was maintained during 15 min of 70° HUT in the post-exercise condition at a HR which was associated with hypotension in the control tilt. The absence of a difference in orthostatic tachycardia between the two experimental conditions implies that factors other than baroreflex control of cardiac responses were altered by exercise. Our observation of a greater FVR during post-exercise HUT compared to control suggests that the responsiveness of vasoconstrictor mechanisms were enhanced by acute intense exercise and contributed significantly to post-exercise maintenance of blood pressure during tilt. However, the specific mechanism(s) responsible for the increased vasoconstriction remains to be identified.

With a cross-over experimental design, we observed no distinguishable difference in the average baseline FVR between the control and exercise conditions, and test-retest comparisons for individual subjects were significantly correlated. These findings support the notion that occlusion plethysmographic measurement of FVR was highly reproducible from day-to-day, and indicates that increased baroreflex response of FVR during HUT following exercise in this study was the result of the exercise treatment rather than some factor associated with methodology or time.

The mechanism by which FVR response during HUT was increased by acute exercise is unclear. Alterations in circulating volume can change cardiopulmonary baroreceptor control of vascular responses (Thompson et al., 1990). Since vascular volume was not altered by the acute exercise in this study, it is unlikely that increased FVR during HUT after exercise could be explained by this mechanism. An alternative explanation for greater post-exercise FVR during tilt might be that exercise elicited a greater response of neural and humoral vasoactive agents such as NE, AVP, and PRA to orthostasis. It is widely documented that orthostasis causes increased plasma levels of NE (Blomqvist and Stone; Convertino, 1987a), AVP (Convertino, 1987a), and PRA (Blomqvist and Stone; Convertino, 1987a; Shvartz et al., 1981) and that these hormone levels are associated with orthostatic tolerance (Shvartz et al., 1981). Elevations in plasma NE, AVP, and PRA in our subjects at the end of tilt

were similar to those reported in paraplegics (Guttmann et al., 1963; Mendelsohn and Johnston, 1971; Poole et al., 1987) and there were no differences in these responses between the exercise and control conditions. Therefore, increased FVR during HUT following acute intense exercise could not be explained by increased levels of these circulating vasoactive hormones. However, the observation that FVR was significantly increased during post-exercise HUT at similar levels of circulating NE, AVP, and PRA may reflect that the exercise bout induced acute vascular receptor hypersensitivity to these neuroendocrine agents. This possibility remains speculative without further investigations designed to pharmacologically assess alterations in vascular receptor response.

Tachycardia mediated by the baroreflexes provides a means to buffer transient changes in arterial blood pressure (Blomqvist and Stone, 1983). Data from both human and animal models have demonstrated that impairment of the carotid-cardiac reflex is associated with less tachycardia and occurrence of orthostatic hypotension during upright posture (Convertino et al., 1990a, 1991; Cowley et al., 1973). A positive correlation has been reported between the degree of impairment of baroreflex function and incidence of syncope during standing in healthy men following prolonged confinement to bed (Convertino et al., 1990a). Similarly, sinoaortic denervated dogs illustrated smaller heart rate increases and greater blood pressure reductions during

upright posture than dogs with intact baroreflexes (Cowley et al., 1973). These results suggest that if reductions in baroreflex function lead to attenuated cardiac responses during an orthostatic challenge, then enhancement of baroreflex sensitivity should result in greater tachycardia and blood pressure stability. Using a neck cuff device, the influence of a single bout of exhaustive exercise on the cardioacceleratory limb of the carotid-cardiac baroreflex was assessed from changes in heart rate from baseline values induced by an elevation of ~40 mmHg in cuff pressure. The consequent 40-mmHg reduction in carotid distending pressure elicited a calculated elevation in heart rate of 5 bpm (from 62 to 67) during control and 7 bpm (from 61 to 68) 24 h post-exercise (Fig. 4). Thus, although increased carotid-cardiac baroreflex sensitivity was relatively large and consistent, it appears unlikely that vagally-mediated alterations in heart rate can completely explain the tachycardia noted during HUT. This raises the possibility that other factors responsible for cardioacceleration, possibly sympathetically-mediated baroreflexes, were influenced by exercise.

We used the unit change in HR to the unit change in SBP (ΔHR/ΔSBP) during phases I, II, and IV of the Valsalva maneuver as an index of integrated arterial baroreflex stimulus-response relationship. The ΔHR/ΔSBP represents an index of non-specific baroreflex control of heart rate since pressure reductions are likely to influence heart rate through interaction of cardiopulmonary, aortic, and carotid

baroreceptor stimulation (Korner et al., 1976; Porth et al., 1984; Smith et al., 1987b; Stegemann et al., 1985; Tatro et al., 1992). Phase I occurs at the onset of the strain and is characterized by an elevation in SBP and a slight baroreflex-mediated bradycardia. The increase in SBP is considered to be mechanical in nature and primarily dependent on the intensity of the maneuver and the blood volume (Stegemann et al., 1985). Therefore, our observation of similar post-exercise phase I $\Delta HR/\Delta SBP$ compared to control is consistent with our finding of no difference in PV between the two experimental conditions.

Phase II of the maneuver is marked by an initial fall in SBP followed by a slight rise (Fig. 3). The fall in blood pressure is accompanied by increases in HR and systemic vasoconstriction. These baroreflex-mediated actions prevent a further decline in blood pressure and facilitate the late phase II increase in pressure (Porth et al., 1984). Our subjects exhibited a substantially greater late phase II rise in SBP in the post-exercise condition compared to control. Since total autonomic blockade markedly attentuated the increase in arterial pressure, the late rise in phase II SBP can be attributed to autonomically-mediated reflex cardioacceleration and vasoconstriction (Korner et al., 1976). If this is true, then our results support the possibility that the 20 mmHg higher SBP measured in our subjects during the post-exercise condition was the result of enhanced autonomic function.

Phase IV represents the post-strain period in which a reduction in HR is observed in conjunction with a rapid elevation in blood pressure. In the control condition, an increase of 44 mmHg in SBP stimulated a 31 bpm decrease in heart rate so that $\Delta HR/\Delta SBP$ was 0.72 beats·mmHg^{-1}. Twenty four hours after maximal exercise, an increase of 47 mmHg in SBP elicited a reduction in heart rate of 44 bpm so that $\Delta HR/\Delta SBP$ was 1.01 beats·mmHg^{-1}. Thus, exercise induced a 40% increase in baroreceptor-cardiac reflex response. The change in HR during this phase of the Valsalva maneuver is thought to be mediated predominantly by sinoaortic baroreceptor cardiac reflex activity (Smith et al., 1987b). Clearly, the reduction in HR observed in the post-exercise condition for a similar BP stimulus was markedly greater than that induced during the control test. Since carotid sinus baroreceptors do not singly contribute to the reflex control of heart rate (Mark and Mancia, 1983), a portion of the enhanced cardiac reflex response during the post-exercise Valsalva strain can be attributed to sensitized aortic baroreceptor control. This is reasonable since aortic baroreceptor-cardiac reflex has a more prominent role than the carotid baroreceptor-cardiac reflex in the control of heart rate (Ferguson et al., 1985; Mancia et al., 1977).

In summary, orthostatic hypotension during 15 min of 70° HUT was eliminated 24 h after completion of a single bout of exhaustive arm execise in paraplegic subjects. Since exercise did not alter vascular volume, plasma levels of

vasoactive hormones, or leg compliance, improved blood pressure stability during HUT must be explained by increased sensitivity of autonomically-mediated baroreceptor reflex control of cardiac and vascular responses. Indeed, maintenance of arterial pressure 24 h following acute intense exercise was associated with a heightened sensitivity of arterial baroreceptor stimulus-cardiac response relationships and greater forearm vascular resistance during HUT.

CHAPTER 5
EFFECT OF A SINGLE BOUT OF EXHAUSTIVE EXERCISE ON INTEGRATED BAROREFLEX FUNCTION AFTER 16 DAYS OF 6° HEAD-DOWN TILT[2]

5.1 Abstract

Altered baroreflex function is a frequently observed consequence of microgravity exposure. Baroreflex function is enhanced in the 24 h following performance of exhaustive, dynamic exercise. Therefore, we tested the hypothesis that one bout of maximal exercise performed 24 h prior to re-ambulation from 16 days of uninterrupted 6° head-down tilt (HDT) could increase the gain of integrated baroreflex function. To do this, heart rate (HR) and blood pressure (MAP) were continually measured during a 15-sec Valsalva maneuver (VM) performed by seven subjects at a controlled expiratory pressure of 30 mmHg before and after two periods of HDT separated by 11 months. On the last day of one HDT period, subjects performed a single bout of maximal cycle ergometry (exercise). Subjects did not exercise after the other HDT period (control). There were no differences in baseline HR (62 vs. 61 bpm; $r = 0.841$, $P = 0.012$) or MAP (87 vs. 89 mmHg; $r = 0.864$, $P = 0.018$) between HDT exposures. The ratio of the change in HR to change in MAP ($\Delta HR/\Delta MAP$)

[2] This research was supported in part by a NASA grant administered under contracts NAS9-611 and NAS10-10285.

during phases II and IV of the VM was used as an index of cardiac baroreflex sensitivity. Baroreflex-mediated vasoconstriction was assessed by measuring the late phase II rise in MAP. Sixteen days of HDT decreased ΔHR/ΔMAP during phase II by 47% (1.5 to 0.8 beats/mmHg; P = 0.002). After completion of exhaustive leg exercise, ΔHR/ΔMAP in phase II exceeded pre-HDT levels (2.3 beats/mmHg), while it remained substantially attenuated in control (0.7 beats/mmHg). There were no distinguishable differences (P > 0.05) in ΔHR/ΔSBP during phase IV between treatments. The late phase II increase of MAP was 71% greater (7 mmHg vs. 2 mmHg; P = 0.041) following exercise compared to control. These observations suggest that global baroreflex control of cardiac chronotropic and vasoconstrictive responses, which are diminished by HDT, are enhanced in the 24 h period after completion of a single bout of maximal exercise.

5.2 Introduction

Altered function of autonomically-mediated cardiovascular reflexes is a frequently observed consequence of exposure to real and simulated weightlessness as suggested by reports of attenuated vagal carotid-cardiac baroreflex gain (Convertino et al., 1990a, 1991, 1992; Eckberg and Fritsch, 1992; Fritsch et al., 1992), increased sensitivity of aortic-cardiac baroreflex (Crandall et al., 1993), and greater vasoconstriction mediated by cardiopulmonary

baroreceptor stimulation (Convertino et al., 1994a) following spaceflight, bedrest, and wheelchair confinement. However, because reflex integrity was assessed in these studies with techniques which isolated each specific reflex arc, only limited conclusions can be drawn regarding effects of microgravity on integrated baroreflex function.

Acute graded exercise to exhaustion has been reported to increase the sensitivity of cardiovascular reflexes and enhance maintenance of blood pressure during the post-exercise recovery period. One bout of maximal exercise increased carotid baroreceptor stimulus-cardiac reflex response by 60 min post-exercise (Somers et al., 1985) and lasted as long as 24 h after exercise (Convertino and Adams, 1991; Convertino et al., 1992). Intense dynamic exercise after 10 days of bedrest returned heart rate (HR), blood pressure, and orthostatic stability to pre-bedrest levels within 2 h of ambulation (Convertino, 1987b), and decreased the incidence of fainting after 6 h of water immersion (Stegemann et al., 1975). These observations suggest that exercise of this type may provide an effective stimulus to counter microgravity-induced alterations of cardiovascular reflex function.

The purpose of this study, therefore, was to test the hypothesis that a single bout of maximal exercise performed 24 h prior to re-exposure to the normal 1-g gravitational environment could reverse integrated baroreflex dysfunction caused by extended exposure to simulated microgravity. Our

results suggest that global baroreflex function, which is diminished by HDT, is enhanced in the 24 h period after completion of a single bout of maximal exercise.

5.3 Methods

Informed consent was obtained from seven healthy, normotensive, nonsmoking men [means (± SE) age 40 ± 2 yr, height 183 ± 2 cm, and weight 81 ± 2 kg] who served as subjects for this investigation. The experimental protocol consisted of two 22-day periods of confinement to the Human Research Facility at NASA-Ames Research Center separated by 11 months. Each period consisted of 4 days of ambulatory control followed by 16 days of 6° head-down tilt and 2 days of post-HDT recovery. All subjects completed a single bout of graded exercise to exhaustion twenty-four hours prior to arising from the head-down posture (exercise condition). Following the other, subjects performed no exercise (control condition). Heart rate and blood pressure responses to the Valsalva maneuver were measured before HDT, on day 15 of HDT, and 30 min prior to returning to the upright posture (day R-1). All Valsalva maneuvers where conducted at the same time of day.

5.4 Results

Valsalva maneuver. Pre-HDT baseline HR (62 ± 1 vs. 61 ± 1 bpm for control and exercise, respectively) and MAP (87 ± 1

vs. 89 ± 1 mmHg for control and exercise, respectively) were not different between conditions and were highly reproducible ($r = 0.841$, $p = 0.018$ and $r = 0.864$, $p = 0.012$ for HR and MAP, respectively). In all experimental conditions, beat-to-beat blood pressure and HR responses elicited by the Valsalva maneuver demonstrated a classic four phase response pattern (Figure 4.2). A characteristic increase in MAP was observed in phase I (point b) and was followed by a biphasic phase II consisting of an early decline in MAP (point c) which was interrupted by a small rise in blood pressure (point d). Phase III was characterized by a rapid decrease in MAP (point e) as a result of termination of the maneuver and was followed by the phase IV blood pressure overshoot (point f).

Mean alterations in systemic pressure and HR during the Valsalva maneuver in each experimental condition are presented in Table 5.1. Figure 5.1 illustrates average HR and MAP responses to the Valsalva maneuver in the control and exercise conditions. Fifteen days of HDT reduced $\Delta HR/\Delta MAP$ during phase II by 47% (1.5 to 0.8 beats/mmHg; $P = 0.002$; Table 1). After completion of exhaustive leg exercise, $\Delta HR/\Delta MAP$ in phase II exceeded pre-HDT levels (2.3 vs. 1.5 beats/mmHg, $P = 0.017$), while it remained substantially attenuated in control (0.7 beats/mmHg; Table 5.1, Figure 5.2A). There were no differences ($P > 0.05$) in $\Delta HR/\Delta SBP$ during phase IV at any time (Table 5.1). The late phase II increase in MAP just prior to the termination of the maneuver

was 71% greater (P = 0.041) following exercise compared to control (Fig. 5.2B).

Table 5.1. Comparison of mean heart rate and blood pressure changes to the Valsalva maneuver between experimental conditions.

	Pre-HDT	HDT Day 15	Day R-1 + Exercise	Day R-1 + Control
Phase II				
ΔHR, bpm	−12 ± 1	−25 ± 1*	−12 ± 1	−29 ± 2*
early ΔSBP, mmHg	18 ± 1	20 ± 1	28 ± 3*	19 ± 2
ΔHR/ΔSBP, b·mmHg^{-1}	1.5 ± .1	0.8 ± .1*	2.3 ± .3*†	0.7 ± .1*
late ΔSBP, mmHg	4 ± 1	1 ± 2	7 ± 1†	2 ± 2
Phase IV				
ΔHR, bpm	25 ± 1	33 ± 3*	29 ± 2	32 ± 2*
ΔSBP, mmHg	−31 ± 1	−35 ± 1	−38 ± 2	−40 ± 2*
ΔHR/ΔSBP, b·mmHg^{-1}	1.2 ± .1	1.1 ± .1	1.3 ± .1	1.3 ± .1

Values are mean ± SE.
* = P < 0.05 compared to pre-HDT
† = P < 0.05 compared to Day R-1 + control

Exercise bout. Final work rate at volitional fatigue averaged 1443 ± 81 kpm·min^{-1} and was attained after a mean time of 16 ± 0.8 min. HR, systolic and diastolic blood pressures at termination averaged 182 ± 3 bpm, 177 ± 6 mmHg, and 77 ± 4 mmHg, respectively.

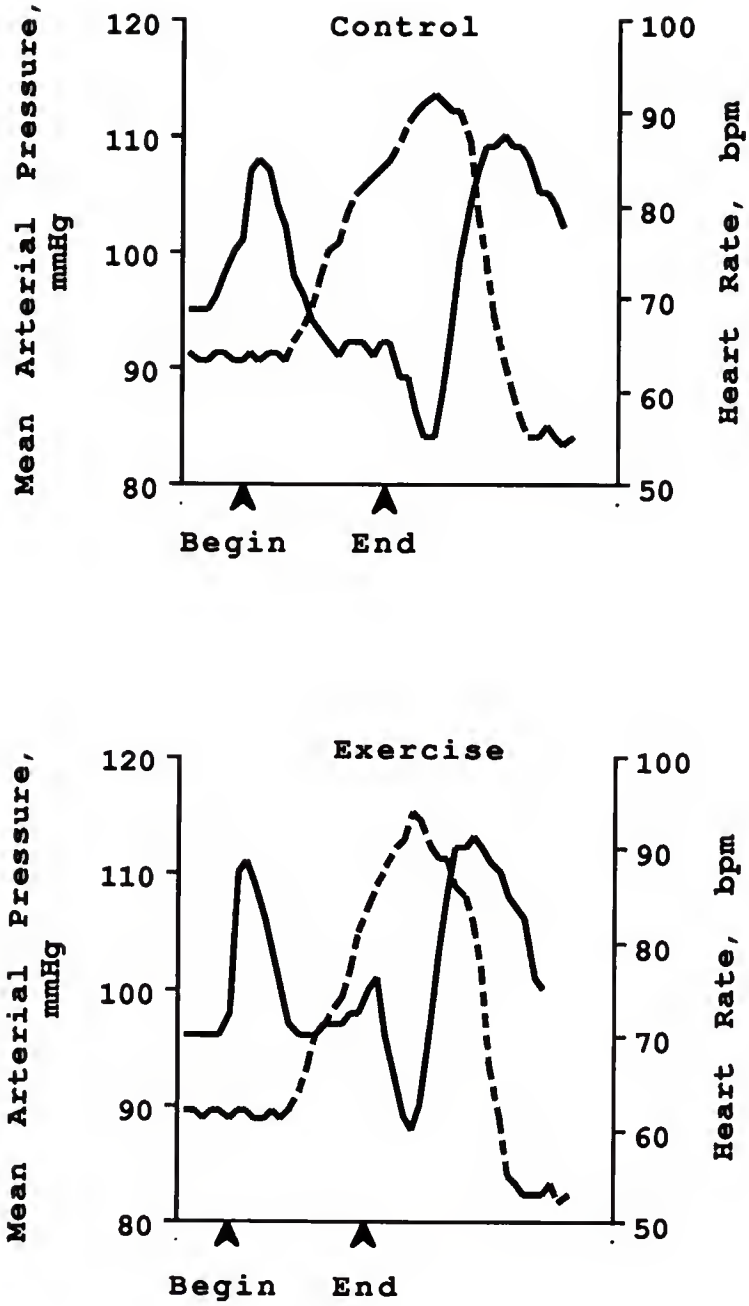

Figure 5.1. Mean HR (dashed line) and MAP (solid line) responses to a 15-s Valsalva maneuver on day R-1 at an expiratory pressure of 30 mmHg in control and post-exercise conditions.

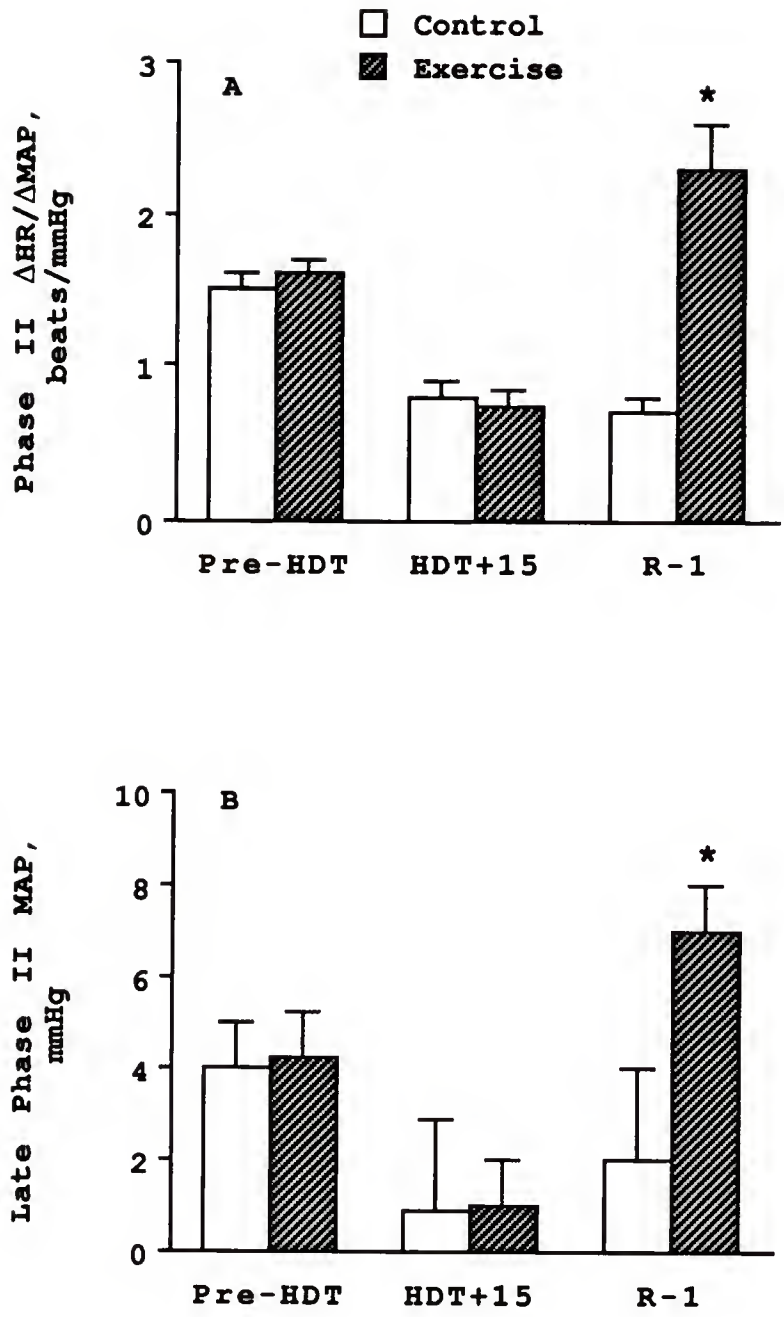

Figure 5.2. Ratio of the unit change in HR to unit change in MAP (ΔHR/ΔMAP) during phase II of the Valsalva maneuver (panel A) and change in MAP from pre-Valsalva baseline during late phase II (panel B) prior to HDT (pre-HDT), after 15 days of HDT (HDT + 15), and just prior to re-ambulation (R-1) for the control (open bars) and exercise conditions (hashed bars). Asterisks indicate $P < 0.05$ compared with corresponding R-1 value.

5.6 Discussion

To test the hypothesis that a single bout of dynamic exercise designed to elicit maximal effort could reverse attenuation of overall baroreflex function following extended exposure to microgravity, we measured HR and blood pressure responses to the Valsalva maneuver in seven subjects who performed a single bout of exhaustive cycle ergometry exercise 24 h prior to re-ambulation from 16 days of uninterrupted 6° HDT. A primary finding of this study was that microgravity attenuated integrated baroreflex function. This observation is consistent with previous measurements of altered baroreflex function following exposure to simulated and actual microgravity (Convertino et al., 1990a, 1992, 1994a; Crandall et al., 1993; Eckberg and Fritsch, 1992; Fritsch et al., 1992). Another novel finding of this study was that after exercise, $\Delta HR/\Delta MAP$ in phase II, an index of cardiac baroreflex sensitivity, exceeded pre-HDT levels while it remained substantially attenuated in the control condition. Additionally, the late phase II increase of MAP was 71% greater following exercise compared to control, indicating that vasoconstrictor responses were augmented after exercise as well. These observations suggest that global baroreflex function, which is diminished by HDT, is enhanced in the 24 h period following completion of a single bout of maximal exercise.

In all experimental conditions, we observed a four-phase HR and blood pressure response pattern typical of the Valsalva maneuver. Although alterations in arterial pressure during phases I and III are due to the application and removal of increased intrathoracic pressure generated by the maneuver (Korner et al., 1976; Porth et al., 1984; Sharpey-Schafer, 1965), HR and blood pressure responses to phases II and IV provide a reliable, non-invasive means by which to assess the integrity of autonomically-mediated cardiac and vascular baroreflex responses (Korner et al., 1976; Sharpey-Schafer, 1965). The ratio describing the unit change in HR to the unit change in MAP ($\Delta HR/\Delta MAP$) during phases II and IV represents an index of non-specific baroreflex control of heart rate since pressure reductions are likely to influence heart rate through interaction of cardiopulmonary, aortic, and carotid baroreceptor stimulation (Korner et al., 1976; Porth et al., 1984). Therefore, by calculating this ratio, we were able to evaluate the effect of extended HDT and maximal exercise on the integrated cardiac baroreflex stimulus-response relationship.

As expected, the phase I increase in arterial pressure was followed by a decline in blood pressure typical of the early portion of phase II. The magnitude of the decrease in arterial pressure and concomitant increase in HR during this portion of the maneuver was influenced by both HDT and maximal exercise. This is clearly illustrated by our observation of a 29-mmHg fall of MAP which stimulated a 19-

bpm increase of HR after HDT in the control condition ($\Delta HR/\Delta MAP$ = 0.7 beats·mmHg^{-1}). Twenty-four hours after maximal exercise, a decrease of 12 mmHg of MAP elicited an increase in heart rate of 28 bpm so that $\Delta HR/\Delta MAP$ was 2.3 beats·mmHg^{-1}. Thus, exercise resulted in a three-fold greater baroreceptor-cardiac reflex response that eliminated hypotension in early phase II with the same tachycardic response (Fig. 5.1).

Although phase II cardioacceleration is the result of parasympathetic withdrawal mediated by arterial baroreflexes (Leon et al., 1970; Sandroni et al., 1991), the mechanism responsible for the increased tachycardia we observed after exercise is unclear. This issue is complicated by uncertainty regarding the identity of the specific baroreceptor population influencing cardiac responses to phase II of the maneuver (Eckberg, 1980). In conscious rabbits, the rise of HR during Valsalva-like respiratory efforts is decreased after dennervation of carotid sinus afferents, but is more attenuated following section of aortic arch afferents suggesting that aortic-cardiac baroreflexes predominate (Blombery and Korner, 1982). Conversely, since in humans transmural pressure across the aorta remains constant during the initial moments of phase II (Sharpey-Schafer, 1965), it is reasonable to suggest that aortic baroreflex influence on cardiac activity is minimal and carotid sinus baroreflexes are responsible for phase II cardiac responses. This possibility is supported by the

observation that aortic arch cross-sectional area was not changed until the mid-point of a Valsalva maneuver performed at 40 mmHg for ~25 s (Greenfield et al., 1967). Therefore, if phase II HR response is carotid sinus baroreflex-mediated, then our observation of accentuated hypotension and minimal cardioacceleration after HDT in the control condition may be attributed to an impairment of the carotid-cardiac baroreflex stimulus-response relationship after prolonged HDT. This is consistent with reports of reduced carotid baroreflex sensitivity and minimal elevation of HR in response to provocative testing following both real (Fritsch et al., 1992) and simulated microgravity (Convertino et al., 1990a, 1991, 1992; Eckberg and Fritsch, 1992). Enhanced function of the efferent limb of this vagally-mediated baroreflex may explain increased phase II tachycardia in the post-exercise condition since heightened sensitivity of this baroreflex has been observed after exhaustive exercise (Convertino and Adams, 1991; Convertino et al., 1992). Thus, our observation of a markedly greater $\Delta HR/\Delta MAP$ after exercise condition supports the possibility that an exercise stimulus acutely enhances the function of baroreceptor-mediated reflexes responsible for hypotensive tachycardia.

During late phase II, elevations in HR and vasoconstriction combine to arrest the decline of arterial pressure caused by prolonged Valsalva straining (Porth et al., 1984; Sharpey-Schafer, 1965). Several investigators (Delius et al., 1972; Sarnoff et al., 1948) have reported

this vasoconstriction results from increased sympathetic activity; an explanation which is consistent with the observation that phentolamine completely eliminated the late phase II increase in MAP (Korner et al., 1976; Sandroni et al., 1991). Since sympathetic outflow is modulated by arterial (Bevegard and Shepherd, 1966; Hagbarth and Vallbo, 1968) and cardiopulmonary (Zoller et al., 1972) baroreflex mechanisms, it is possible that the 5-mmHg greater late phase II MAP in the post-exercise condition may be explained by increased sensitivity of baroreflex-mediated vasoconstrictor reponses. Our current observation of increased late phase II blood pressure also confirms our previous finding in which we noted that paraplegic subjects exhibited increased forearm vascular resistance during 70° head-up tilt 24 h after completion of maximal armcrank exercise compared to a control condition (see Chapter 4). In the paraplegic subjects, heightened vasoconstrictor response could not be explained by differences in levels of circulating pressor agents between experimental conditions since neither baseline nor post-tilt levels of plasma renin activity, norepinephrine, and arginine vasopressin were dissimilar. It is possible that exhaustive exercise may have increased end-organ receptor density and/or sensitivity to vasoactive substances since similar bouts of activity have acutely increased ß-adrenergic receptor response characteristics (Butler et al., 1983), but the effect exercise has on alpha-adrenergic receptor responses in the 24 h post-exercise recovery period remains to be

clarified. However, because peripheral resistance increased within ~12 s of the onset of hypotension, the mechanism responsible for increased vasoconstriction following exhaustive exercise is most likely neural.

Phase IV occurs immediately after cessation of the Valsalva strain and is characterized by a rapid rise in arterial pressure as venous return is enhanced and an increased cardiac output is ejected into an arterial bed still constricted as a result of phase II vasoconstriction (Porth et al., 1984; Sharpey-Schafer, 1965). The rapid increase in arterial pressure is accompanied by elevations of vagal tone mediated by the sinoaortic baroreflexes (Eckberg, 1980; Sandroni et al., 1991) and a period of sympathetic silence (Delius et al., 1972). In the present study, phase IV $\Delta HR/\Delta MAP$ was not altered by HDT nor by performance of exhaustive exercise. HDT has been reported to heighten sensitivity of the isolated aortic-cardiac baroreflex response (Crandall et al., 1993), but resulted in a marked reduction in responsiveness of the carotid-cardiac baroreflex (Convertino et al., 1990a, 1991, 1992; Eckberg and Fritsch, 1992; Fritsch et al., 1992). Therefore, this suggests that the magnitude of increase in isolated aortic baroreflex gain might be offset by the decrease in carotid baroreflex function such that the net effect of microgravity on integrated baroreflex response in phase IV was obscured. Likewise, despite its effect to enhance isolated carotid-cardiac baroreflex response after HDT (Convertino et al.,

1992), there was no effect of acute, maximally intense exercise on total integrated reflex response in phase IV since there was no difference in ΔHR/ΔMAP between control and exercise conditions. A possible explanation for this observation is that, while exercise induced an increased sensitivity of the carotid-cardiac baroreflex, it may have reversed aortic baroreflex function to baseline, ultimately resulting in a similar ΔMAP/ΔHR between conditions. This possibility remains speculative, however, until the influence of exhaustive exercise on isolated aortic baroreflex function is determined. Regardless, our current results suggest that integrated sinoaortic baroreflex control of HR responds quite differently to microgravity and exercise than do responses elicited by isolated baroreflexes.

Since hypovolemia is a consequence of prolonged microgravity exposure (Convertino, 1994; Convertino et al., 1994a) and acute, maximally intense exercise has been demonstrated to increase blood volume (Gillen et al., 1991), it is possible that alterations in circulating blood volume may explain the dissimilarity of responses to the Valsalva maneuver between the two experimental conditions. Indeed, redistribution of vascular volume induced by postural changes has been observed to alter cardiovascular dynamics during the Valsalva maneuver (Stegemann et al., 1985; Ten Harkel et al., 1990), but it is unclear how changes in absolute blood volume influence responses to Valsalva straining. Thus, blood volume inequalities may have influenced Valsalva responses.

However, we observed enhanced cardiac and vascular responses to the Valsalva strain following performance of a bout of dynamic arm exercise without differences in plasma volume between the exercise and control conditions (see Chapter 4). These results suggest that exercise can cause alterations in HR and blood pressure responses to the maneuver similar to those induced in the present study and independent of changes in vascular volume. These data, together with those demonstrating that acute alterations of blood volume had no effect on the carotid-cardiac baroreceptor stimulus-response relationship (Thompson et al., 1990), support the notion that exhaustive exercise may enhance neural cardiovascular control mechanisms independent of volemic state.

In summary, sixteen days of HDT diminished integrative baroreflex function as indicated by cardiac and vascular responses to the Valsalva maneuver. Completion of a single bout of exhaustive leg exercise 24 h prior to arising from HDT resulted in an increased $\Delta HR/\Delta MAP$ and elevated arterial pressure during phase II of the maneuver compared to the control condition. Neither HDT nor exercise elicited a change in the phase IV $\Delta HR/\Delta MAP$ relationship. These observations suggest that integrated baroreflex control of cardiac and vascular function is altered by microgravity and exercise in a way which may not be reflected by isolated baroreflex stimulus-response relationships.

CHAPTER 6
MAINTENANCE OF LBNP TOLERANCE 24 HOURS AFTER MAXIMAL EXERCISE FOLLOWING 16 DAYS OF SIMULATED MICROGRAVITY EXPOSURE[3]

6.1 Abstract

We tested the hypothesis that one bout of maximal exercise performed 24 h prior to re-ambulation from prolonged simulated microgravity would improve blood pressure stability during an orthostatic challenge. To do this, heart rate (HR), mean arterial blood pressure (MAP), leg volume (LV), norepinephrine (NE), and forearm vascular resistance (FVR) were measured during lower body negative pressure (LBNP) to pre-syncope in 7 subjects immediately prior to re-ambulation from 16 days of 6° head-down tilt (HDT) under two experimental conditions: 1) after maximal cycle ergometry performed 24 h prior to returning to the upright posture (exercise) and 2) without exercise (control). Plasma volume (PV), leg compliance (LC), and carotid-cardiac baroreflex (CCB) sensitivity were also measured under both conditions. After 16 days of HDT, the reduction of LBNP tolerance time from pre-HDT levels was greater ($P = 0.041$) in the control condition (-2.0 ± 0.2 min) compared to the exercise condition (-0.4 ± 0.2 min). At the point of pre-syncope following HDT,

[3] This work was supported in part by a NASA grant administered under contracts NAS9-611 and NAS10-10285.

FVR was higher ($P = 0.041$) following exercise (101 ± 9 PRU) compared to control (84 ± 6 PRU). MAP and HR at pre-syncope were similar in both conditions as was LV. PV and CCB sensitivity were reduced ($P < 0.05$) by 16% and 26% respectively following 16 days of HDT in both conditions, but returned to pre-HDT levels in the exercise condition. LC measured after HDT was not different from pre-HDT levels in either condition. Maintenance of orthostatic tolerance post-exercise was associated with greater vasoconstriction, restored PV, and enhanced CCB sensitivity.

6.2 Introduction

Compromised blood pressure control during an orthostatic challenge has been observed after exposure to microgravity and its analogs (Convertino et al., 1990a; Bungo and Johnson, 1983; Hoffler, 1977). Although the cause of orthostatic hypotension appears to be caused by an array of factors and their interactions, decreased plasma volume (Blomqvist and Stone, 1983; Bungo et al., 1985), loss of vascular tone in the lower extremities (Convertino et al., 1989a; Hoffler, 1977; Luft et al., 1976), and altered orthostatic tachycardia and baroreflex function have been observed following spaceflight (Fritsch et al., 1992), prolonged bedrest (Convertino et al., 1990a, 1992; Eckberg and Fritsch, 1992), and chair confinement (Convertino et al., 1991). Since blood pressure is the product of heart rate, stroke volume, and

peripheral resistance, these observations suggest that loss of circulating volume, decreased baroreceptor function, increased vascular compliance, and altered autonomic function may contribute alone or in combination to the predipostion of an individual to orthostatic intolerance following microgravity exposure.

Several investigations have provided evidence that a single bout of exhaustive dynamic exercise expands plasma volume and enhances the function of mechanisms responsible for blood pressure stability. A 10% expansion in plasma volume was induced 24 h after the performance of maximal leg exercise in ambulatory subjects (Gillen et al., 1991). An increased gain of the carotid-cardiac baroreflex has been observed 60 min after completion of one bout of graded exercise (Somers et al., 1985) and for as long as 24 h post-exercise (Convertino and Adams, 1991). A single bout of high intensity exercise at the end of 10 days of bedrest restored heart rate, blood pressure, and orthostatic stability to pre-bedrest levels within 2 h of ambulation (Convertino, 1987b), reversed fainting episodes after 6 h of water immersion (Stegemann et al., 1975), and re-sensitized carotid baroreflex function after 7 days of head-down tilt bedrest (Convertino et al., 1992). These observations suggest that acute, high intensity exercise provides a physiological stimulus capable of increasing the reserve of response mechanisms associated with blood pressure regulation. We tested the hypothesis that a single bout of dynamic maximal

exercise would increase the sensitivity of the carotid-cardiac baroreflex, restore plasma volume, and enhance blood pressure stability during an orthostatic challenge following 16 days of 6° head-down tilt (HDT).

6.3 Methods

Seven healthy, nonsmoking, normotensive men [mean (±SE) age of 40 ± 2 yr, height of 183 ± 2 cm, and weight of 81 ± 2 kg, VO_2max 2.9 ± 0.4 $L \cdot min^{-1}$] gave their written consent to serve as subjects for this investigation after they had been informed of all procedures and risks. The experimental protocol consisted of a total of 22 days confinement in the Human Research Facility at NASA-Ames Research Center including 4 days of ambulatory control followed by 16 days of 6° head-down tilt and 2 days of post-HDT recovery. All measurements where conducted at the same time of day and in the same sequence during both HDT exposure periods. Plasma volume, and leg compliance were measured on the fourth pre-HDT control day (C-4), the last day of HDT (BR-16), and just prior to arising from the HDT posture (R-1). On days C-4 and R-1, each subject underwent a test of orthostatic tolerance using a graded LBNP protocol to pre-syncope. Each subject performed an acute bout of graded exercise designed to elicit maximal effort twenty-four hours prior to the R-1 measurements of plasma volume, leg volume, baroreflex function, NE, and hemodynamic responses to LBNP during one of

the HDT exposures (exercise condition). Following the other, subjects performed no exercise (control condition).

6.4 Results

Responses to LBNP. Measurements of pre-HDT baseline MAP, HR, NE, FVR, LC, LV, and LBNP tolerance times were highly reproducible between treatment conditions (Table 6.1). As a result, these variables were averaged and expressed as a single value.

Table 6.1. Reproducibility of baseline measures between experimental conditions.

	Control	Exercise	*r*	P value
LBNP time, min	48 ± 1	47 ± 1	0.914	0.034
MAP, mmHg	87 ± 3	89 ± 2	0.864	0.012
HR, bpm	57 ± 3	58 ± 3	0.841	0.018
NE, $pg \cdot ml^{-1}$	135 ± 13	138 ± 14	0.859	0.062
PV, ml	3743 ± 164	3718 ± 170	0.881	0.090
FVR, pru	39 ± 4	40 ± 4	0.769	0.044
CCB, $ms \cdot mmHg^{-1}$	2.7 ± .8	2.8 ± .8	0.880	0.009
LC, $ml \cdot mmHg^{-1}$	3.0 ± .4	2.9 ± .5	0.881	0.009
LV, $ml \cdot 100ml^{-1}$	1.8 ± .2	1.7 ± .2	0.787	0.063

Values are mean ± SE.

Following HDT, time to pre-syncope was reduced by 2 ± 0.2 min (P = 0.041) in the control condition, averaging 47.4

min before and 45.4 min after HDT. When subjects performed exercise 24 hours prior to the end of HDT, LBNP tolerance time was maintained or improved in 3 subjects with the overall effect being that time to pre-syncope was unchanged (47.8 ± 1 to 47.3 ± 1 min, P = 0.451; Figure 6.1a). One subject (subject MG, Figure 6.1b) was excluded from the LBNP tolerance time analysis because he represented an outlying response with a reduction of 18 min in LBNP tolerance time following 16 days of bedrest without exercise. In contrast, when maximal exercise was performed 24 hours before the end of HDT, his LBNP tolerance was actually improved by more than 1 min (Fig. 6.1b).

As depicted in Figure 6.2a, MAP at pre-syncope was similar for all pre- and post-HDT LBNP tests. HR response to LBNP was increased following HDT (P = 0.013), but there was no difference between conditions (Figure 6.2B). Baseline FVR was increased on day R-1 compared to day C-4 (39 ± 4 to 50 ± 7 and 52 ± 9 PRU for days C4, R-1+Exercise and R-1+Control, respectively), but increases where not different between conditions. During LBNP, FVR at pre-syncope increased to 101.6 ± 3 PRU following exercise which was greater (P = 0.046) than FVR measured in the control condition (84.3 ± 6 PRU; Fig. 6.3). Post-HDT vasoconstrictor reserve, measured as the difference between FVR at 0 mmHg LBNP and FVR at pre-syncope, tended to be decreased from pre-HDT values in the control condition (47 ± 6 to 35 ± 10 pru; P = 0.068), but was unchanged following exercise (53 ± 11 pru).

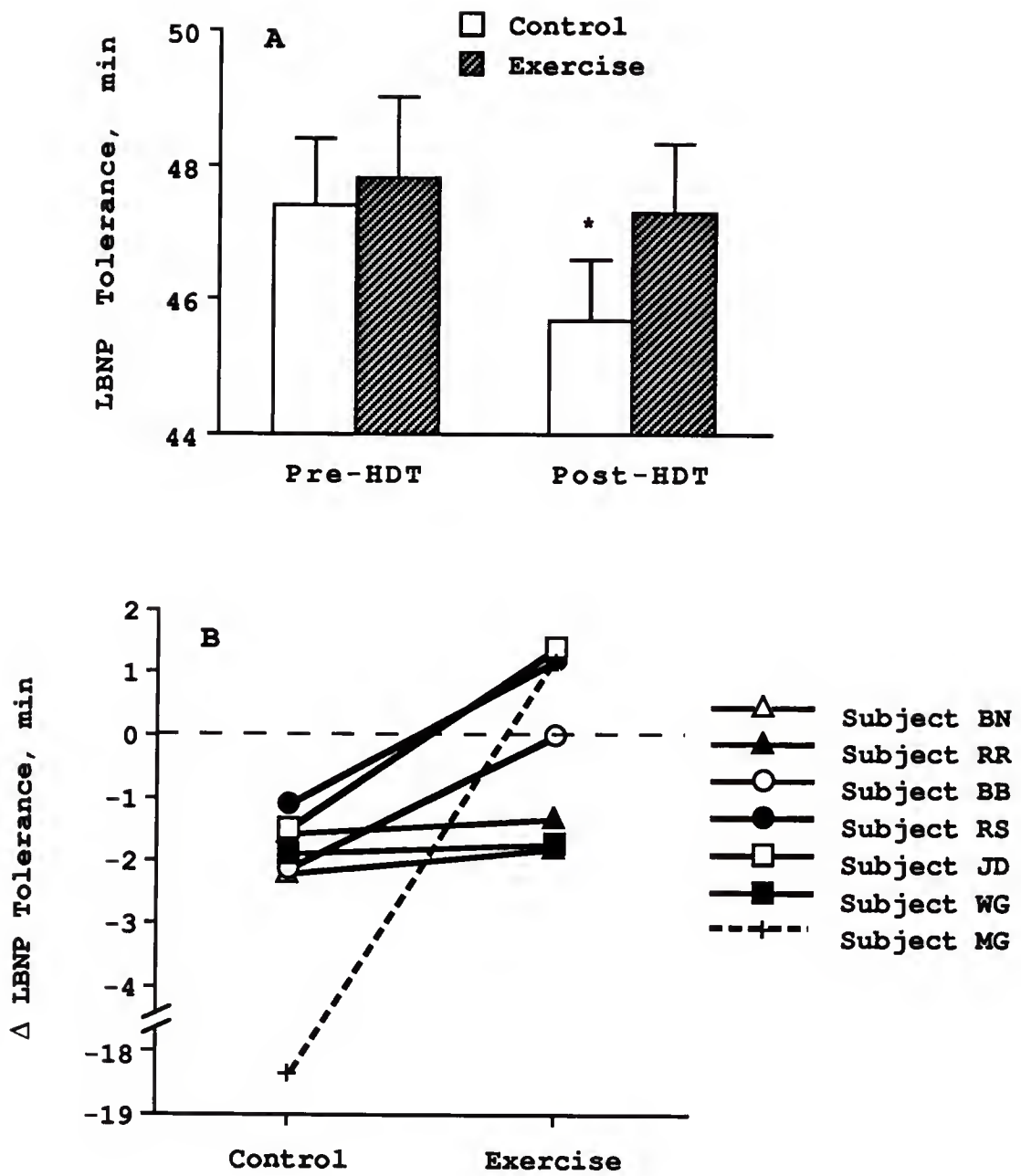

Figure 6.1. Panel A: Maximal LBNP tolerance time before (pre-HDT) and after 16 days of HDT (post-HDT). Asterisk indicates P = 0.041 from pre-HDT. Panel B indicates the difference in LBNP tolerance time from pre-HDT for each of the seven subjects.

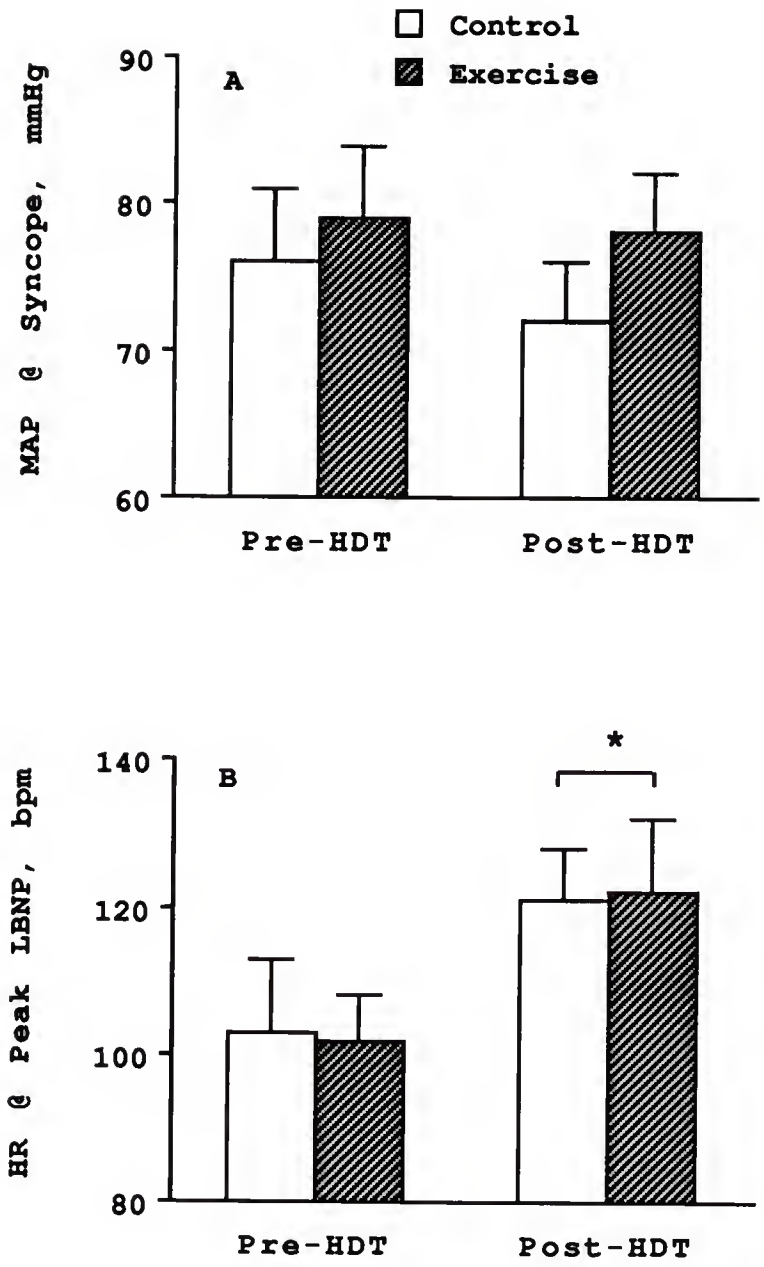

Figure 6.2. Mean arterial pressure (panel A) and heart rate (panel B) responses at peak LBNP. In Panel B, asterisks indicate P = 0.013 vs. pre-HDT HR.

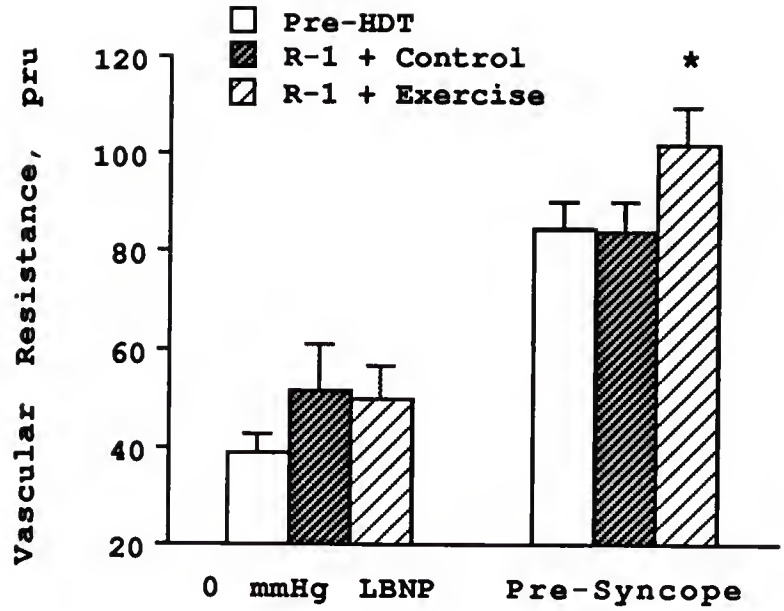

Figure 6.3. Range of forearm vascular resistance responses to LBNP before and after 16 days HDT in control and exercise conditions. Asterisk indicates P = 0.046 compared to FVR at pre-syncope in R-1 control condition.

Plasma NE concentration measured at peak LBNP was elevated over baseline levels both before and after 16 days of HDT (155 ± 25 to 518 ± 85 $pg \cdot ml^{-1}$; P = 0.003), but LBNP-induced increase in NE was not different between treatments. Likewise, there was a greater change in leg volume at pre-syncope measured during LBNP on the final day of HDT compared to pre-HDT (1.8 to 2.2 $ml \cdot 100ml^{-1}$; P = 0.057), but the increase was not dissimilar between conditions.

Leg Compliance. Resting left calf compliance following HDT was not different (P = 0.699) than that measured prior to 16 days of HDT in either condition (2.9 ± 0.4 vs. 3.1 ± 0.4 $ml \cdot mmHg^{-1}$).

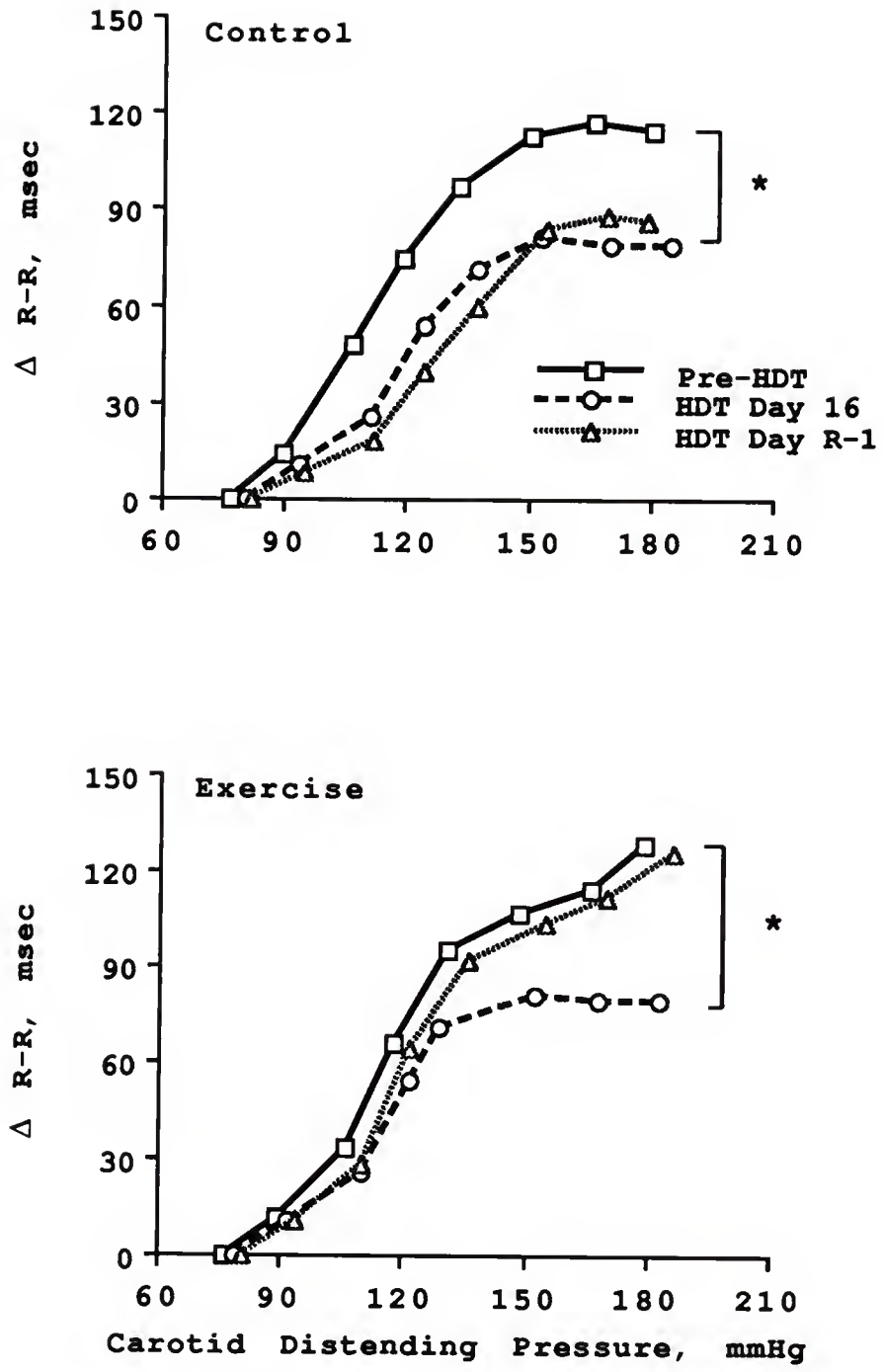

Figure 6.4. Carotid-cardiac baroreflex stimulus-response relationships before (pre-HDT), on day 16 (HDT Day 16), and just prior to re-ambulation from 16 days of HDT (HDT Day R-1) in the control (upper panel) and exercise (lower panel) conditions. Asterisks indicated P = 0.05 vs. pre-HDT values.

Carotid-Cardiac Baroreflex Response. Carotid-cardiac baroreflex sensitivity was reduced (P = 0.05) by 26% following 16 days of HDT in both conditions (2.8 to 2.0 msec·mmHg^{-1}; Fig. 6.4). A single bout of maximal exercise performed on day 16 of HDT restored the baroreflex gain to pre-HDT levels (2.8 to 2.6 msec·mmHg^{-1}), while it remained substantially attenutated in the control condition (2.8 msec·mmHg^{-1} to 2.0 msec·mmHg^{-1}; Fig. 6.4).

Plasma Volume. By day 16 of HDT, plasma volume had decreased by 16% (P = 0.001) in both conditions (3730 ± 173 to 3130 ± 111 ml). However, PV was completely restored to pre-HDT levels (3838 ± 126 ml) following performance of maximal exercise on the afternoon of HDT day 16, whereas circulating volume remained reduced (P = 0.007) from pre-HDT in the control condition (3301 ± 133 ml).

Exercise bout. For the seven subjects, final work rate at volitional fatigue averaged 1443 ± 81 kpm·min^{-1} and was attained after a mean time of 16 ± 0.8 min. HR, and systolic and diastolic blood pressures at termination averaged 182 ± 3 bpm, 177 ± 6 mmHg, and 77 ± 4 mmHg respectively.

6.5 Discussion

We measured heart rate, mean aterial pressure, forearm vascular resistance, leg volume, and norepinephrine responses during LBNP to pre-syncope in 7 subjects 24 h after performance of maximal supine cycle ergometry to test the

hypothesis that exhaustive exercise can increase the reserve capacity of blood pressure control mechanisms and minimize or eliminate hypotension during orthostasis following 16 days of 6° head-down tilt. The unique finding of this study was that orthostatic tolerance was maintained at pre-HDT levels when subjects performed acute exercise prior to returning to the upright posture. Post-exercise maintenance of orthostatic tolerance was associated with elevated MAP during LBNP, enhanced carotid-cardiac baroreflex sensitivity, greater vasoconstriction, and restoration of plasma volume. Improved blood pressure control occurred without differences in orthostatic tachycardia, leg volume or compliance, or circulating levels of NE between conditions.

Orthostatic responses. Orthostatic tolerance was defined as the ability to maintain arterial pressure ≥ 80 mmHg during progressive LBNP with the absence of pre-syncopal symptoms such as dizziness and nausea. As expected, LBNP elicited increased heart rate, peripheral resistance, leg volume, and norepinephrine to levels comparable to those observed by others who employed similar techniques to test orthostatic responses (Blomqvist and Stone, 1983; Convertino, 1987a; Davies et al., 1976; Raven et al, 1984; Sather et al., 1986; Smith and Raven, 1986). It has been suggested that tolerance to progressive LBNP can be partially explained by differences in pre-LBNP cardiac output reserves and higher compensatory increases in peripheral resistance to a given orthostatic stress (Convertino et al., 1994b; Sather et al.,

1986). Consistent with this finding is our observation of a greater disposition for hypotension and shorter time to pre-syncope in the control condition where subjects exhibited smaller elevations in systemic peripheral resistance during LBNP and continued decrements in plasma volume following HDT compared to the exercise condition.

Carotid-cardiac baroreflex function. The 26% reduction in carotid-cardiac baroreflex sensitivity we observed following HDT was not unexpected since depressed carotid-cardiac baroreflex function is a frequently observed consequence of real (Fritch et al., 1992) and simulated (Convertino et al., 1990a, 1992; Eckberg and Fritsch, 1992) microgravity. Persistent depression of reflex sensitivity has been noted for a minimum of 5 (Convertino et al., 1990a) and for as long as 10 days (Fritch et al., 1992) following return to a 1-*g* environment suggesting a slow time course of baroreflex re-sensitization upon re-exposure to full terrestrial gravity. In contrast, our results indicate that microgravity-induced reductions in carotid baroreflex sensitivity are completely reversed within 24 h by performance of a single bout of exhaustive dynamic exercise. Thus, it appears that the time course of re-sensitization is dramatically altered by exercise.

During an orthostatic challenge, the carotid baroreflex contributes to the defense of arterial pressure by mediating orthostatic tachycardia (Blomqvist and Stone, 1983; Mancia and Mark, 1983). Impairment of the carotid stimulus-cardiac

response relationship has been associated with an attenuated heart rate response and higher incidence of orthostatic hypotension during standing in healthy men following prolonged HDT (Convertino et al., 1990a) and in sinoaortic denervated animals (Cowley et al., 1973). Based on these observations, it appears tenable that if diminished reflex function leads to orthostatic hypotension, then enhancement of baroreflex sensitivity should result in greater tachycardia and blood pressure stability. Indeed, our observation of a 2-min reduction of LBNP tolerance time in the control condition was associated with a 26% reduction in carotid-cardiac baroreflex sensitivity following 16 days HDT. In contrast, carotid baroreflex gain and LBNP time to pre-syncope were essentially no different from pre-HDT values when subjects performed a bout of exhaustive dynamic exercise 24 h before undergoing an orthostatic challenge.

To determine the effect of heightened carotid-cardiac baroreflex sensitivity on the cardioacceleratory limb of the reflex, changes in heart rate from baseline stimulated by an elevation of ~40 mmHg in neck cuff pressure were measured. A 40-mmHg reduction in carotid distending pressure elicited a calculated elevation in heart rate of 5 bpm (from 52 to 57) during control and 6 bpm (from 55 to 61) 24 h post-exercise. These calculations, combined with our observation that LBNP reduced MAP and increased HR to a similar degree in both experimental conditions, suggest that greater tolerance for orthostasis following exercise cannot be completely explained

by enhanced carotid baroreflex-mediated tachycardia. However, these results must be interpreted with caution since isolated baroreflex function may not reflect integrated baroreflex control of cardiac responses.

Vascular responses. In the absence of differences in heart rate elevation during LBNP, maintenance of orthostatic tolerance 24 h after exercise may possibly be explained by reduced venous pooling and increased arterial resistance. An inverse relationship between leg vascular compliance and LBNP tolerance has been reported by Luft et al. (1976) suggesting that the capacity to maintain venous return, cardiac output, and systemic arterial pressure during an orthostatic challenge can be influenced by the amount of blood pooled in the lower extremities. In the present study, however, there was no difference in resting leg compliance nor leg volume at pre-syncope between control and exercise conditions, eliminating the possibility that maintenance of LBNP tolerance following exercise could be explained by differences in the quantity of blood sequestered in the legs.

Progressive application of sub-atmospheric pressure to the lower body elicited large increases of forearm vascular resistance at the point of pre-syncope. Elevation of vasoconstriction during LBNP was substantially greater following performance of maximal exercise (Fig. 6.3) compared to control. This finding is not without precedent since we previously observed that vasoconstriction was greater at the end of 15 min of 70° head-up tilt 24 h after completion of

exhaustive exercise compared to that noted at the end of tilt not preceeded by maximal exercise (see chapter 4). Our observation that improved orthostatic tolerance is associated with greater systemic resistance was also in agreement with previous findings (Convertino et al., 1994b; Sather et al., 1986). Interestingly, it has been suggested that just prior to the point at which the orthostatic stimulus is sufficient to elicit hypotension, compensatory mechanisms are usually operating at maximal capacity (Rowell, 1986). If this is true, elevation of FVR by 53 PRU from baseline to pre-syncope following exercise compared to a 35 PRU elevation in the control condition fails to support the hypothesis advanced by Myers and Honig (1969) who proposed the existence of an upper limit to the vasoconstrictive capacity of the vasculature. Although we noted an increased baseline FVR following HDT, this does not appear to explain greater FVR at pre-syncope in the exercise condition because vascular resistance was similarly increased in the control condition. An alternative explanation for increased FVR following exercise is that exhaustive leg ergometry increased the maximal vasoconstrictive reserve, thus providing a greater capacity to increase peripheral resistance to defend mean arterial pressure during orthostasis. This notion is supported by our observation that the difference in FVR measured between 0 mmHg and peak LBNP, an index of vasoconstrictor reserve, tended to be greater in the exercise condition compared to

control. This possibility remains speculative, however, and awaits future research.

Circulating volume and vasoactive hormones. Circulating volume and vasoactive hormones have a profound effect on arterial pressure during orthostasis (Blomqvist and Stone, 1983). Increased incidence of orthostatic hypotension has been widely observed in hypovolemic subjects due to reductions in venous return and compromised cardiopulmonary baroreflex control of systemic resistance (Blomqvist and Stone, 1983; Convertino et al., 1994b; Thompson et al., 1990). Consistent with other reports (Gillen et al., 1991), we observed that exhaustive, dynamic leg exercise elicited an increase in circulating volume which completely reversed the 16% reduction of plasma volume induced by 16 days of head-down tilt. It is possible that improved blood pressure maintenance during LBNP 24 h after exercise was associated with enhanced cardiac filling (Frank-Starling effect). Furthermore, predisposition for earlier onset of pre-syncope in the control condition may be related to an increased slope of the stimulus-response relationship of the cardiopulmonary baroreflex resulting from HDT-induced hypovolemia (Convertino et al., 1994a). These data suggest that hypovolemia leads to greater reflex response of FVR for a given decrease in central venous pressure (Convertino et al., 1994a) and is consitent with observations that acute (Thompson et al., 1990) and chronic (Blamick et al., 1988) plasma volume reductions were associated with greater vasoconstriction

compared to a normovolemic state. Increased slope of the cardiopulmonary barorelfex stimulus-response relationship has been interpreted to represent a greater utilization of vasoconstrictive reserves such that maximal vasoconstrictive capacity is attained at a lesser reduction in central blood volume (Thompson et al., 1990). Therefore, it is reasonable to suggest that reduced LBNP tolerance time in the control condition is related to hypovolemia-induced reduction of vasoconstrictive reserve. Conversely, it appears as if maximal exercise provided a stimulus which restored vasoconstrictive reserve following HDT, possibly by its effects on plasma volume.

An additional explanation for increased post-exercise FVR during orthostasis might be that exercise elicited a greater response of neural and humoral vasoactive agents such as norepineprine. It is widely documented that plasma levels of NE (Blomqvist and Stone, 1983; Convertino, 1987a) are increased during orthostasis and that circulating levels of this hormone influence orthostatic tolerance (Shvartz et al., 1981). In the present study, since elevations of plasma NE at pre-syncope were similar between exercise and control conditions, increased FVR during LBNP following exhaustive exercise cannot be explained by increased levels of NE. However, our observation that FVR was significantly increased during post-exercise LBNP at similar levels of circulating NE seen in the control condition may reflect an exercise-induced hypersensitivity of vascular receptors to this neuroendocrine

agent. This possibility, however, remains speculative without further investigations designed to pharmacologically assess alterations in vascular receptor response.

In summary, orthostatic tolerance was maintained at pre-HDT levels when subjects performed acute exercise 24 h prior to returning to the upright posture after 16 days of 6° head-down tilt. Maintenance of orthostatic tolerance following exercise was associated with enhanced carotid-cardiac baroreflex sensitivity, greater vasoconstriction at pre-syncope, and restoration of plasma volume. In addition, improved blood pressure control occurred without alterations in orthostatic tachycardia, leg volume or compliance, or circulating levels of norepinephrine.

CHAPTER 7
SUMMARY AND CONCLUSIONS

7.1 Summary

Microgravity induces alterations in the functional capacity of mechanisms responsible for blood pressure regulation. A single bout of exhaustive exercise enhances performance of various components of the blood pressure control system. As illustrated in Figure 7.1, exposure to low gravitational environments has a profound effect on high and low pressure baroreflex function, leg compliance, autonomic nervous system function, and plasma volume. It is hypothesized that decreased capacity of these basic blood pressure control mechanisms leads to increased incidence of orthostatic intolerance upon re-exposure to full terrestrial gravity. Figure 7.2 identifies the specific components of the orthostasis-stimulus cardiovascular-reflex response cascade which are enhanced in the 24 hours following completion of acute, maximally intense exercise. By comparing Figures 7.1 and 7.2, it is clear that exhaustive exercise provides a stimulus which increases functional capacity of blood pressure regulatory mechanisms attenuated by microgravity, thus potentially reversing decrements in orthostatic tolerance.

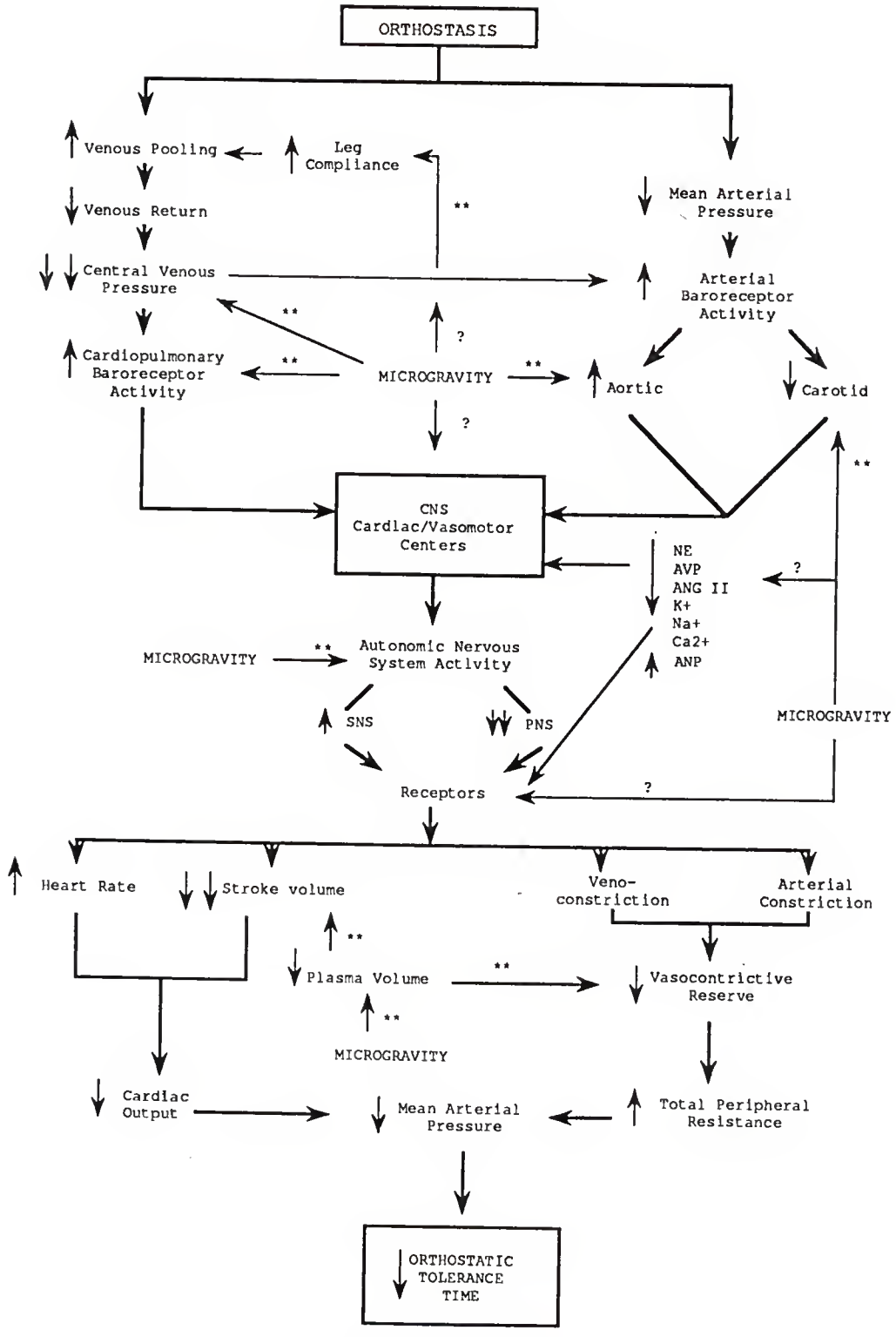

Figure 7.1. Reflex responses to orthostasis following microgravity exposure. Asterisks indicate where altered gravitational environments impact mechanisms responsible for blood pressure control. Questions marks identify where microgravity may potentially exert an effect, but confirmation awaits future research.

The purpose of the experiments included in this dissertation was to investigate the association between mechanisms of blood pressure regulation altered by microgravity exposure and maximal exercise. We did this by examining the effects of an acute exercise stimulus on basic mechanisms associated with blood pressure homeostasis during an orthostatic challenge. This approach allowed us to systematically investigate the effects of both microgravity and maximal exercise on individual components of the blood pressure regulatory system. To do this, plasma volume, leg vascular responses, arterial baroreflex sensitivity, plasma levels of vasoactive hormones, and cardiovascular responses to changes in intra-thoracic pressure and orthostatic stress were measured with and without exercise treatment in 17 subjects exposed to simulated microgravity. The results of these experiments suggest acute, exhaustive exercise provides a specific physiological stimulus that can attenuate or reverse microgravity-induced cardiovascular adaptations associated with orthostatic hypotension.

In Study 1 (Chapter 4), we tested the hypothesis that maximal exercise would enhance blood pressure maintenance during head-up tilt in subjects whose blood pressure control system had been impaired due to prolonged restriction to the seated posture. The results indicated that orthostatic hypotension during head-up tilt was eliminated 24 h after completion of exhaustive arm execise. Since exercise did not alter vascular volume, plasma levels of vasoactive hormones,

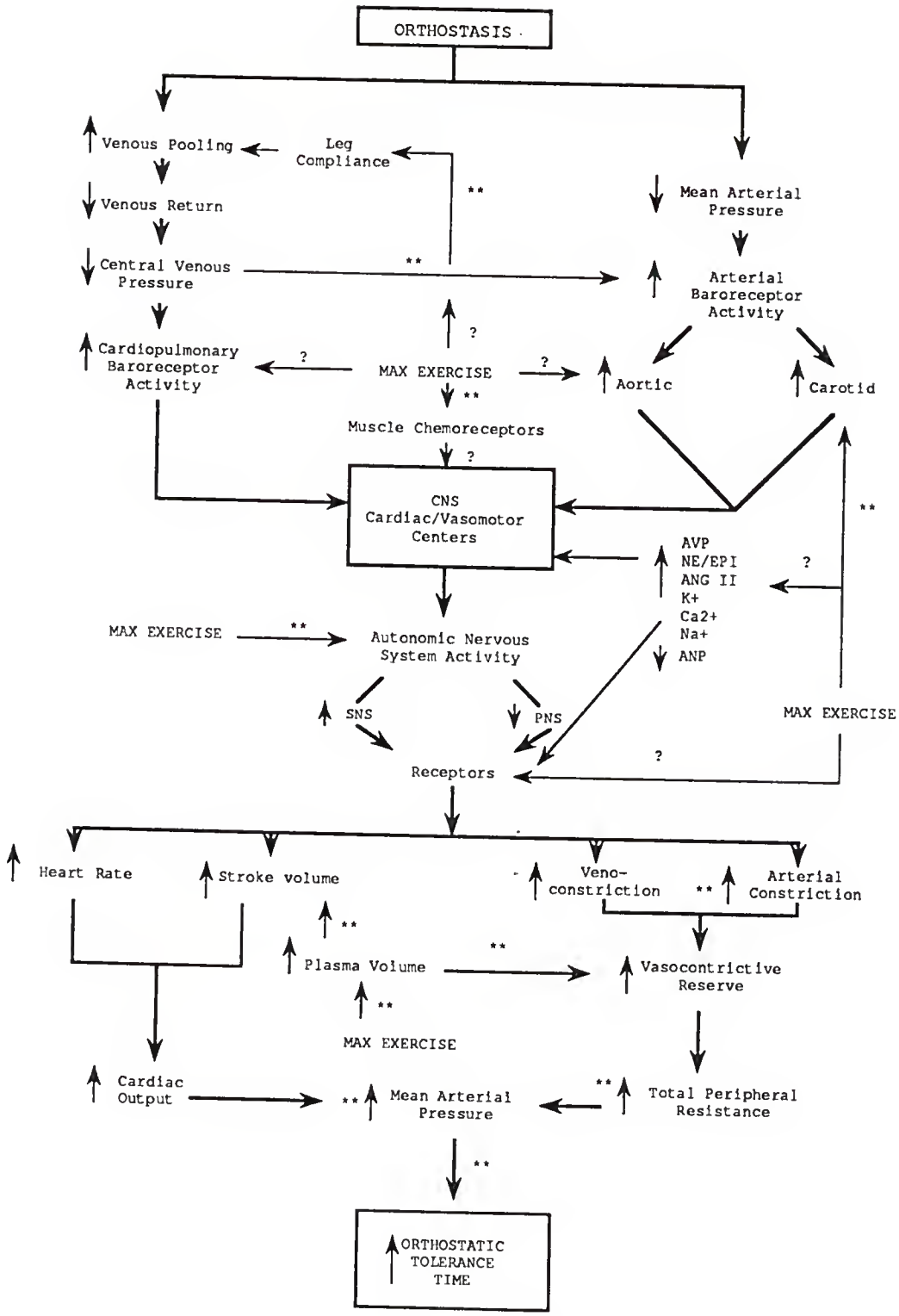

Figure 7.2. Specific components of the orthostasis-stimulus cardiovascular-reflex response cascade which are enhanced following completion of acute, maximally intense exercise are indicated by asterisks. Question marks suggest areas for future research.

nor leg compliance, improved blood pressure stability during tilt must be explained by increased sensitivity of autonomically-mediated baroreceptor reflex control of cardiac and vascular responses. Indeed, maintenance of arterial pressure 24 h following acute, high intensity exercise was associated with a heightened sensitivity of arterial baroreceptor stimulus-cardiac response relationships and greater forearm vascular resistance during head-up tilt.

Although the results from Study 1 provided evidence that elimination of orthostatic hypotension during head-up tilt was associated with performance of exhaustive arm exercise, inferences drawn from these results may be limited by the unique subject population and our failure to test true orthostatic tolerance. Therefore, Study 2 was designed to further examine the relationship between microgravity- and maximal exercise-induced changes in blood pressure control by measuring maximal orthostatic tolerance in ambulatory subjects who had been exposed to 16 days of 6° head-down tilt. We hypothesized that one bout of exhaustive exercise performed just prior to re-ambulation would be effective in attenuating or reversing decrements in blood volume, baroreflex sensitivity, cardiovascular function, and orthostatic stability following prolonged head-down tilt and enhance blood blood pressure control during severe orthostasis. The results of this study are presented in Chapters 5 and 6.

Chapter 5 discussed alterations in integrated baroreflex function and how these interactions are modified by head-down tilt and maximal leg exercise. The results indicated that 16 days of HDT resulted in diminished function of the integrative baroreflexes as indicated by peripheral resistance changes and ΔHR/ΔMAP relationship measured during the Valsalva maneuver. Twenty four hours after completion of exhaustive leg exercise, ΔHR/ΔMAP and the increase in arterial pressure during phase II exceeded pre-HDT levels while it remained substantially attenuated in the control condition. These observations suggest that both cardiac and vascular baroreflex function is enhanced following completion of maximal exercise.

Chapter 6 presented evidence which supported our hypothesis that a single bout of dynamic maximal exercise would increase the sensitivity of the carotid-cardiac baroreflex, restore plasma volume, enhance blood pressure stability, and protect orthostatic tolerance during progressive LBNP following 16 days of 6° head-down tilt. The results of this experiment indicated that orthostatic tolerance was maintained at pre-HDT levels when subjects performed acute exercise 24 h prior to returning to the upright posture after HDT. Maintenance of orthostatic tolerance following exercise was associated with enhanced carotid-cardiac baroreflex sensitivity, greater vasoconstriction at pre-syncope, and restoration of plasma volume. In addition, improved blood pressure control

occurred without alterations in orthostatic tachycardia, leg volume or compliance, or circulating levels of norepinephrine.

From the experiments summarized above, we can conclude that physiological adaptations to microgravity include reduced vascular volume, attenuated function of high and low pressure baroreflexes, diminished autonomic function, and decreased tolerance to orthostasis as manifested by reduced time to pre-syncope during upright tilt or lower body negative pressure (Fig. 7.1). Conversely, adaptations occurring in the 24 h following completion of acute, exhaustive exercise include expanded plasma volume, enhanced sensitivity of the carotid-cardiac baroreflex, heightened autonomic nervous system function, and maintenance of orthostatic tolerance (Fig. 7.2). Thus, the present results clearly indicate that physiological adaptations to acute, maximally intense exercise enhance the functional capacity of the mechanisms responsible for maintaining blood pressure homeostasis.

However, the impact of a single bout of intense exercise on other components of the blood pressure control system remains unclear (see Fig. 7.2). Is the heightened vascular resistance we observed during orthostasis following exercise a result of an enhanced stimulus-response relationship of the cardiopulmonary baroreflex or can it be attributed to increased vascular responsiveness (sensitivity) to circulating vasoactive agents such as NE, AVP, or Ang II?

Futhermore, what is the effect of exercise-induced changes in the hormonal or ionic environment on central cardiovascular control centers? Also, it has been recently observed that prolonged exposure to microgravity leads to heightened aortic-cardiac sensitivity. Based on our observations that exhaustive exercise reversed microgravity-induced attentuations of carotid baroreflex function, can it be assumed that a similar stimulus will return the sensitivity of the aortic baroreflex to pre-exposure levels? Finally, since exercise of this nature undoubtedly stimulates afferent signals from muscle chemoreceptors, questions remain regarding their role in modulation of blood pressure control following maximal dynamic exercise.

Clearly, acute, maximally intense exercise appears to provide a specific physiological stimulus that can enhance blood pressure regulation. The present results identify that heightened function of these basic mechanisms contribute to improved blood pressure homeostasis during an orthostatic challenge.

7.2 Conclusions

1. Adaptations to microgravity included attenuated carotid-cardiac stimulus-response relationship, reduced plasma volume, and decreased orthostatic tolerance. Acute, maximally intense exercise heightened the sensitivity of the carotid-cardiac baroreflex and increased plasma

volume. These findings are consistent with other reports.

2. Elimination of orthostatic hypotension during 15 min of 70° head-up tilt following maximal arm exercise was associated with increased forearm vascular resistance and baroreflex sensitivity and independent of changes in blood volume, circulating levels of vasoactive hormones, and leg compliance.
3. Integrative baroreflex function, as indicated by the $\Delta HR/\Delta MAP$ and vasoconstrictor responses during the Valsalva maneuver, was altered by microgravity. These responses where restored following performance of maximal exercise.
4. LBNP time to pre-syncope was reduced by approximately 2 min in all subjects following 16 days of HDT. When maximal exercise was performed, time to pre-syncope following HDT was indistinguishable from pre-HDT levels.
5. Maintenance of orthostatic tolerance was associated with greater plasma volume, heightened sensitivity of the vagally-mediated carotid baroreflex, and increased maximal forearm vascular resistance. Time to pre-syncope was independent of differences in orthostatic tachycardia, leg volume, leg compliance, or circulating NE.
6. Maximal exercise appears to provide a stimulus to increase or maintain vasoconstrictor reserve, possibly through its effects on vascular volume.

7. Questions remain regarding the influence of exhaustive exercise on the aortic and cardiopulmonary baroreflexes, vascular reponsiveness to circulating vasoactive agents, and central modulation of afferent input stimulated by exercise.

REFERENCES

Aars H. Effects of altered smooth muscle tone on aortic diameter and baroreceptor activity in anesthetized rabbits. Circ Res 28:254-262, 1971

Aars H. Effects of noradrenaline on activity in single aortic baroreceptor fibers. Acta Physiol Scand 83:335-343, 1981

Abboud FM, Eckberg DL, Johannsen UJ, and Mark AL. Carotid and cardiopulmonary baroreceptor control of splanchnic and forearm vascular resistance during venous pooling in man. J Physiol Lond 286:173-184, 1979

Abboud FM and Mark AL. Cardiac baroreceptors in circulatory control in humans. In: Cardiac Receptors, edited by R Hainesworth, C Kidd, and RJ Linden, Cambridge University Press, Oxford, p437-462, 1979

Ackermann U, Khanna J, and Irizawa TG. Atrial natriuretic factor alters autonomic interactions in the control of heart rate in conscious cats. Can J Physiol Pharmacol 66:930-936, 1988

Adams WE. The Comparative Morphology of the Carotid Body and Carotid Sinus. Thomas, Springfield, IL, p.1-259, 1958

Addison WHF. The extent of carotid pressoreceptor area in the cat as indicated by its special elastic tissue wall. Anat Record 88:418-419, 1944

Angell-James J. The effects of changes of extramural "intrathoracic" pressure on aortic arch baroreceptors. J Physiol Lond 214:89-103, 1971

Angell-James JE and Lumley JSP. The effects of carotid endarterectomy on the mechanical properties of the carotid sinus and carotid sinus nerve activity in atherosclerotic patients. N Engl J Med 271:820-832, 1964

Alexander N. Plasma volumes and hematocrits in rats with chronic sinoaortic denervation hypertension. Am J Physiol 236:H92-H95, 1979

Atchison DJ and Ackermann U. Influence of atrial natriuretic peptide on autonomic control of heart rate. Am J Physiol 258 (Regulatory Integrative Comp Physiol 27) R718-R723, 1990

Bader H. The anatomy and physiology of the vascular wall. In: Handbook of Physiology. Circulation, sec. 2, vol. II, edited by WF Hamilton and P Dow, American Physiological Society, Washington, DC, p865-889, 1963

Bagshaw RJ and Fischer JM. Morphology of the carotid sinus in the dog. J Appl Physiol 31:198-202, 1971

Bagshaw RJ and Peterson, LH. Sympathetic control of mechanical properties of canine carotid sinus. Am J Physiol 222:254-262, 1967

Bainbridge PA. Influence of venous filling upon rate of the heart. J Physiol Lond 50:65-84, 1915

Ballermann BJ and Brenner B. Role of atrial peptides in body fluid homeostasis. Circ Res 58:619-630, 1986

Barazanji MW and Cornish KG. Carotid and aortic baroreceptor control of heart rate in conscious monkey. Am J Physiol 253 (Heart Circ Physiol 22): H811-H817, 1987.

Berry CA. Medical legacy of Skylab as of May 9, 1974: The manned Skylab missions. Aviat Space Environ Med 47:418-424, 1976

Bevegard S, Castenfors J, and Lindblad LE. Effect of changes in blood volume distribution on circulatory variables and plasma renin activity in man. Acta Physiol Scand 99:237-245, 1977a

Bevegard S, Castenfors J, Lindblad LE, and Tranesjo J. Blood pressure and heart rate regulating capacity of the carotid sinus during changes in blood volume distribution in man. Acta Physiol Scand 99:300-312, 1977b

Bevegard BS and Shepherd JT. Circulatory effects of stimulating the carotid stretch receptors in man at rest and during exercise. J Clin Invest 45:132-142, 1966

Billman GE, Dickey DT, Teoh KK, and Stone HL. Effects of central venous blood volume shifts on arterial baroreflex control of heart rate. Am J Physiol 241 (Heart Circ Physiol 10): H571-H575, 1981

Bishop VS, Hasser EM, and Nair UC. Baroreflex control of renal nerve activity in conscious animals. Circ Res 61 [Suppl. I]:I-76-I-81, 1987

Bishop VS, Lombardi F, Malliani A, Pagani M, and Recordati G. Reflex sympathetic tachycardia during intravenous infusions in chronic spinal cats. Am J Physiol 230:25-29, 1976

Bishop VS, Shade RS, and Haywood JR. Hormonal modulation of baroreceptor reflexes. In: Baroreceptor Reflexes. edited by: PB Persson and HR Kirchheim, Springer-Verlag, New York, p226-236, 1991

Bjurstedt H, Rosenhamer G, and Tyden G. Cardiovascular responses to changes in carotid sinus transmural pressure in man. Acta Physiol Scand 94:497-505, 1977

Blamick CA, Goldwater DJ, and Convertino VA. Leg vascular responsiveness during acute orthostasis following simulated weightlessness. Aviat Space Environ Med 59:40-43, 1988

Blombery PA and Korner PI. Role of aortic and carotid sinus baroreceptors on Valsalva-like vasoconstrictor and heart rate reflexes in the conscious rabbit. J Auton Nerv Syst 5: 303-315, 1982

Blomqvist CG and Stone HL. Cardiovascular adjustments to gravitational stress. In: Handbook of Physiology: The Cardiovascular System. Peripheral Circulation and Organ Blood Flow, part. 2, vol. 3, edited by Shepherd JT and Abboud FM, American Physiological Society, Bethesda, MD, p1025-1063, 1983

Blomqvist CG, Nixon JV, Johnson RL, and Mitchell JH. Early cardiovascular adaptation to zero gravity simulated by head-down tilt. Acta Astronautica 7:543-553, 1980

Bock P and Gorgas K. Fine structure of baroreceptor terminals in the carotid sinus of guinea pigs and mice. Cell Tiss Res 170:95-110, 1976

Boehmer RD. Continuous real-time, non-invasive monitor of blood pressure: Penaz methodology applied to the finger. J Clin Monit 3:282-287, 1987

Boss J and Green JH. The histology of the common carotid baroreceptor areas of the cat. Circ Res 4:12-17, 1956

Bronk DW and Stella G. Afferent impulses in the carotid sinus nerve I. The relation of the discharge from single end organs to arterial blood pressure. J Cell Comp Physiol 1:113-120, 1932

Brooks VL and Reid IA. Interaction between angiotensin II and the baroreceptor reflex in the control of adrenocorticotropic hormone secretion and heart rate in conscious dogs. Circ Res 58:816-828, 1986

Brown, AM. Receptors under pressure. Circ Res 46:1-10, 1980

Brown E, Goei JS, Greenfield ADM, and Plassaras GC. Circulatory responses to simulated gravitational shifts of blood in man induced by exposure of the body below the iliac crests to sub-atmospheric pressure. J Physiol Lond 183:607-627, 1966

Brown MR and Fisher LA. Glucocorticoid suppression of the sympathetic nervous system and adrenal medulla. Life Sci 39:1003-1012, 1986

Buckey JC, Lane LD, Plath G, Gaffney FA, Baisch F, and Blomqvist CG. Effects of dead-down tilt for ten days on the compliance of the leg. Acta Physiol Scand 144:53-60, 1992

Bungo MW, Charles JB and Johnson PC. Cardiovascular deconditioning during space shuttle flight and the use of saline as a countermeasure to orthostatic intolerance. Aviat Space Environ Med 56:985-990, 1985

Bungo MW and Johnson, PC, Jr. Cardiovascular examination and obeservations of deconditioning during the space shuttle orbital test flight program. Aviat Space Environ Med 54:1001-1004, 1983

Butler J, Kelly JG, O'Malley K and Pidgeon F. ß-Adrenoreceptor adaptation to acute exercise. J Physiol 344: 113-117, 1983

Butler GC, Xing HC, Northey DR, and Hughson RL. Reduced orthostatic tolerance following 4 h head-down tilt. Eur J Appl Physiol 62:26-30, 1991

Castenfors J and Sjostrand T. Circulatory control via vagal afferents IV. Integration of the central control mechanisms in the circulatory adaptation to variations of blood volume. Acta Physiol Scand 87:188-198, 1973

Chapleau MW and Abboud FM. Determinants of sensitization of carotid baroreceptors by pulsatile pressure in dogs. Circ Res 65:566-577, 1989

Chapleau MW, Hajduczok G, and Abboud FM. Pulsatile activation of baroreceptors causes central facilitation of baroreflex. Am J Physiol 256 (Heart Circ Physiol 25): H1735-H1741, 1989

Chapleau MW, Heesch CM, and Abboud FM. Prevention or attenuation of baroreceptor resetting by pulsatility during elevated pressure. Hypertension (suppl. III) 9:III-137-III-141, 1987

Chaplin H, Mollison PL, and Vetter H. The body/venous hematocit ratio: Its consistency over a wide hematocrit range. J Clin Invest 32:1309-1316, 1953

Chen RYZ, Matteo RS, Fan F-C, Schuellsler GB, and Chien S. Resetting of baroreflex sensitivity after induced hypotension. Anesthesiology 56:29-35, 1982

Chobanian AV, Little RD, Tercyak A, and Blevins P. The metabolic and hemodynamic effects of prolonged bedest in normal subjects. Circulation 49:551-559, 1974

Coleridge HM, Coleridge JCG, Kaufman MP, and Dangel A. Operational sensitivity and acute resetting of aortic baroreceptors in dogs. Circ Res 48:676-684, 1981

Coleridge HM, Coleridge JCG, Poore EM, Roberts AM, and Schultz HD. Aortic wall properties and baroreceptor behavior at normal arterial pressure and in acute hypertensive resetting in dogs. J Physiol Lond 350:309-326, 1984

Coleridge JCG and Kidd C. Reflex effects of stimulating baroreceptors in the pulmonary artery. J Physiol Lond 166:197-210, 1963

Convertino VA. Aerobic fitness, endurance training, and orthostatic intolerance. In: Exercise and Sports Science Reviews, edited by KB Pandolf and JO Hollozy, Williams and Wilkins, Baltimore, MD, 223-259, 1987a

Convertino VA. Potential benefits of maximal exercise just prior to return from weightlessness. Aviat Space Environ Med 58:568-572, 1987b

Convertino VA. Physiological adaptations to weightlessness: effects on exercise and work performance. In: Exercise and Sports Science Reviews, edited by KB Pandolf and JO Hollozy, Williams and Wilkins, Baltimore, MD, 119-166, 1990

Convertino VA. Effects of exercise and inactivity on intravascular volume and cardiovascular control mechanisms. Acta Astronautica 24:1-7, 1992

Convertino VA. Exercise and adaptation to microgravity environments. In: Handbook of Physiology: Adaptation to the Environment, American Physiological Society, Bethesda, MD, 1994 (in press)

Convertino VA and Adams WC. Enhanced vagal baroreflex response during 24 h after acute exercise. Am J Physiol (Regulatory Integrative Comp Physiol) 260:R570-R575, 1991

Convertino VA, Adams WC, Shea JD, Thompson CA, and Hoffler GW. Impairment of carotid-cardiac vagal baroreflex in wheelchair-dependent quadriplegics. Am J Physiol 260 (Regulatory Integrative Comp Physiol 29):R576-R580, 1991

Convertino VA, Brock PJ, Keil LC, Bernauer EM, and Greenleaf JE. Exercise training-induced hypovolemia: Role of plasma albumin, renin, and vasopressin. J Appl Physiol: Respirat Environ Exercise Physiol 48:665-669, 1980

Convertino VA, Doerr DF, Eckberg DL, Fritsch JM, and Vernikos-Danellis J. Head-down bed rest impairs vagal baroreflex responses and provokes orthostatic hypotension. J Appl Physiol 68:1458-1464, 1990a

Convertino VA, Doerr DF, Flores JF, Hoffler GW, and Buchanan P. Leg size and muscle functions associated with leg compliance. J Appl Physiol 64:1017-1021, 1988

Convertino VA, Doerr DF, Guell A, and Marini JF. Effects of acute exercise on attenuated vagal baroreflex function during bedrest. Aviat Space Environ Med 63:999-1003, 1992

Convertino VA, Doerr DF, Ludwig DL, and Vernikos J. Effect of simulated microgravity on cardiopulmonary baroreflex control of forearm vascular resistance. Am J Physiol (Regulatory Integrative Comp Physiol): 1994a, (in press)

Convertino VA, Doerr DF, Mathes KL, Stein SL, and Buchanan P. Changes in volume, muscle compartment, and compliance of the lower extremities in man following 30 days of exposure to simulated microgravity. Aviat Space Environ Med 60:653-658, 1989a

Convertino VA, Doerr DF, and Stein SL. Changes in size and compliance of the calf after 30 days of simulated microgravity. J Appl Physiol 66:1509-1512, 1989b

Convertino, VA and Engelke KA. Restoration of plasma volume induced by a single bout of maximal exercise after 16 days of simulated microgravity. Aviat Space Environ Med 65:1993

Convertino VA, Mathes KL, Lasley ML, Tomaselli CM, Frey MAB, and Hoffler GW. Hemodynamic and hormonal responses to lower body negative pressure in men with varying profiles of strength and aerobic power. Eur J Appl Physiol 67: 492-498, 1994b

Convertino VA, Thompson CA, Eckberg DL, Fritsch JM, Mack GW, and Nadel ER. Baroreflex responses and LBNP tolerance following exercise training. Physiologist 33:S40-S41, 1990b

Cornish KG, Barazanji MW, Yong T, and Gilmore JP. Volume expansion attenuates baroreflex sensitivity in the conscious nonhuman primate. Am J Physiol 257 (Regulatory Integrative Comp Physiol 26): R595-R598, 1989

Cowley AW, Liard JF, and Guyton AC. Role of the baroreceptor reflex in daily control of arterial pressure and other variables in dogs. Circ Res 32:564-576, 1973

Crandall CG, Engelke KA, Convertino VA, and Raven PB. Aortic baroreflex control of heart rate following 15 days of simulated microgravity. FASEB J 7:A666, 1993

Davies R, Slater JDH, Forsling ML, and Payne N. The response of arginine vasopressin and plasma renin to postural change in normal man, with observations on syncope. Clin Sci Mol Med 51:67-274, 1976

Delius W, Hagbarth K-E, Hongell A, and Wallin GB. Maneuvers affecting sympathetic outflow in human muscle nerves. Acta Physiol Scand 84: 82-94, 1972

DiCarlo SE, Stahl LK, Hasser EM, and Bishop VS. The role of vasopressin in the pressor response to bilateral carotid occlusion. J Autonom Nerv Syst 27:1-10, 1989

Donald DE and Edis AJ. Comparison of aortic and carotid baroreflexes in the dog. J Physiol Lond 218:423-429, 1971

Dorward PK, Andresen MC, Burke SL, Oliver JR, and Korner PI. Rapid resetting of the aortic baroreceptors in the rabbit and its implications for short term and longer term reflex control. Circ Res 50:427-439, 1982

Dorward PK, Riedel W, Burke SL, Gipps J, Korner PI. The renal sympathetic baroreflex in the rabbit. Circ Res 57:618-633, 1985

Doyle JT, Wilson JS, Estes EH, and Warren JV. The effect of intravenous infusions of physiological saline solution on the pulmonary arterial and pulmonary capillary pressure in man. J Clin Invest 30:345-352, 1951

Dujardin JP. Baroreceptor reflex sensitivity after acute blood volume expansion in anesthetized dogs. Experientia Basel 36:428-429, 1980

Ebert TJ. Baroreflex responsiveness is maintained during isometric exercise in humans. J Appl Physiol 61:797-803, 1986

Ebert TJ and Cowley AW. Atrial natriuretic factor attenuates carotid baroreflex-mediated cardioacceleration in humans. Am J Physiol 254 (Regulatory Integrative Comp Physiol 23): R590-R594, 1988

Eckberg DL. Adaptation of the human carotid baroreceptor cardiac reflex. J Physiol Lond 269:561-589, 1977

Eckberg DL. Parasympathetic cardiovascular control in human disease: a critical review of methods and results. Am J Physiol 239 (Heart Circ Physiol 8): H581-H593, 1980

Eckberg DL and Eckberg ML. Human sinus node responses to repetitive, ramped carotid baroreceptor stimuli. Am J Physiol 242:H638-H644, 1982

Eckberg DL and Fritsch JM. Human autonomic responses to actual and simulated weightlessness. J Clin Pharmacol 31:951-955, 1991

Eckberg DL and Fritsch JM. Influence of ten day head-down bedrest on human carotid baroreceptor-cardiac reflex function. Acta Physiol Scand 144:69-76, 1992

Eckberg DL, Abboud FM, and Mark AL. Modulation of carotid baroreflex responsiveness in man: Effects of posture and propranolol. J Appl Physiol 41:383-387, 1976

Eckberg DL, Convertino VA, Fritsch JM, and Doerr DF. Reproducibility of human vagal carotid baroreceptor-cardiac reflex responses. Am J Physiol 263 (Regulatory Integrative Comp Physiol 32): R215-R220, 1992

Eckberg DL, Drabinski M, and Braunwald E. Defective parasympathetic control in patients with heart disease. N Engl J Med 285:877-883, 1971

Eckberg DL, Fletcher GF, and Braunwald E. Mechanism of prolongation of the R-R interval with electrical stimulation of carotid sinus nerves in man. Circ Res 30: 131-138, 1972

Eiken O, Convertino VA, Doerr DF, Dudley GA, Morariu G, and Mejkavic IB. Characteristics of the carotid baroreflex in man during normal and flow-restricted exercise. Acta Physiol Scand 144:325-331, 1992

Elisberg EI. Heart rate response to the Valsalva maneuver as a test of circulatory integrity. J Am Med Assoc 186: 200-205, 1963

Engelke KA, Shea JD, Doerr DF, and Convertino VA. Enhanced carotid-cardiac baroreflex response and elimination of orthostatic hypotension 24 hours after acute exercise in paraplegics. Paraplegia 30:872-879, 1992

Epstein M. Cardiovascular and renal effects of head-out water immersion in man. Application of the model in the assessment of volume homeostasis. Circ Res 39:619-628, 1976

Epstein SE, Beiser GD, Stampfer M, and Braunwald E. Role of venous system in baroreceptor-mediated reflexes in man. J Clin Invest 47:139-152, 1968

Epstein M, DeNunzio AG, and Loutzenhiser RD. Effects of vasopressin administration on the diuresis of water during immersion in normal humans. J Appl Physiol: Respirat Environ Exercise Physiol 51:1384-1387, 1981

Epstein M, Pins DS, and Miller M. Suppression of ADH during water immersion in normal man. J Appl Physiol 38:1038-1044, 1975

Epstein M and Saruta T. Effect of water immersion on renin-aldosterone and renal sodium handling in normal man. J Appl Physiol 31:368-374, 1971

Ernsting J and Parry DJ. Some observations on the effects of stimulating the stretch receptors in the carotid artery of man. J Physiol Lond 135:45P-46P, 1957

Ferguson DW, Abboud FM, and Mark AL. Relative contribution of aortic and carotid baroreflexes to heart rate control in man during steady-state and dynamic increases in arterial pressure. J Clin Invest 76:2265-2274, 1985

Ferrari AU, Daffonchio A, Sala C, Gerosa S, and Mancia G. Atrial natriuretic factor and arterial baroreceptor reflexes in unathesthetized cats. Hypertension 15:162-167, 1990

Ferrario CM, Barnes KL, Diz DI, Block CH, and Averill DD. Role of area postrema pressor mechanisms in the regulation of arterial pressure. Can J Physiol Pharmacol 65:1591, 1987

Fink GD, Haywood JR, Bryan WJ, Packwood W, and Brody MJ. Central site for pressor action of blood-bourne angiotensin in rat. Am J Physiol 239 (Regulatory Integrative Comp Physiol 8): R358-R361, 1980

Fischer CL, Johnson PC, and Berry CA. Red blood cell and plasma volume changes in manned space flight. J Am Med Assoc 200:579-583, 1967

Fisher LA. Central autonomic modulation of cardiac baroreflex by corticotropin releasing factor. Am J Physiol 256 (Heart Circ Physiol 25): H949-H955, 1989

Fisher LA and Brown MR. Corticotropin-releasing factor and angiotensin II: Comparison of CNS actions to influence neuroendocrine and cardiovascular function. Brain Res 296:41-47, 1984

Floras JS. Sympathoinhibitory effects of atrial natriuretic factor in normal humans. Circulation 81:1860-1873, 1990

Floras JS, Aylward PE, Abboud FM, and Mark AL. Inhibition of muscle sympathetic nerve activity in humans by arginine vasopressin. Hypertension 10:409-416, 1987

Fortney SM, Dussack L, Rehbein T, Wood M, and Steinmann L. Effect of prolonged LBNP and saline ingestion on plasma volume and orthostatic responses during bed rest. In: Proceedings of the First Joint NASA Cardiopulmonary Workshop, edited by SM Fortney and AR Hargens, NASA Conference Publications, Houston, TX, p61-70, 1991

Fritsch JM, Charles JB, Bennett BS, Jones MM, and Eckberg DL. Short-duration spaceflight impairs human carotid baroreceptor-cardiac reflex responses. J Appl Physiol 73:664-671, 1992

Fritsch JM, Rea RF, and Eckberg DL. Carotid baroreflex resetting during drug-induced arterial pressure changes in humans. Am J Physiol 256 (Regulatory Integrative Comp Physiol 22): R549-R553, 1989

Garner MG, Phippard AF, Fletcher PJ, MacLean JM, Duggin GG, Horvath JS, and Tiller DJ. Effects of angiotensin II on baroreflex control of heart rate in conscious baboons. Hypertension 10:628-634, 1987

Gauer OH and Henry JP. Circulatory basis of fluid volume control. Physiol Rev 43:423-481, 1963

Gillen CM, Lee R, Mack GW, Tomaselli CM, Nishiyasu T and Nadel ER. Plasma volume expansion in humans after a single intense exercise protocol. J Appl Physiol 71:1914-1920, 1991

Goldman WF and Saum WR. A direct excitory action of catecholamines on rat aortic baroreceptors *in vitro*. Circ Res 55:18-30, 1984

Goldstein RE, Beiser GD, Stampfer M, and Epstein SE. Impairment of the autonomically mediated heart rate control in patients with cardiac dysfunction. Circ Res 36:571-578, 1975

Green JH. Further baroreceptor areas associated with the common carotid artery of the cat. J Physiol, Lond 123:41P, 1954

Greenfield JC, Cox RL, Hernandez RR, Thomas C, and Schoonmaker FW. Pressure-flow studies in man during the Valsalva maneuver with observations on the mechanical properties of the ascending aorta. Circulation 35: 653-661, 1967

Greenleaf JE, Convertino VA, and Mangseth GR. Plasma volume during stress in man: osmolality and red cell volume. J Appl Physiol: Respirat Environ Exercise Physiol 47:1031-1038, 1979

Greenleaf JE, Greenleaf CJ, Van Derveer D, and Dorchak KJ. Adaptation to prolonged bedrest in man: A compendium of research. NASA Technical Memorandum X-3307, Moffett Field, CA, p1-180, 1976

Greenleaf JE, Juhos LT, and Young HL. Plasma lactic dehydrogenase activities in men during bed rest with exercise training. Aviat Space Environ Med 56:193-198, 1985

Greenleaf JE, Silverstein L, Bliss J, Langenheim V, Rossow H, and Chao C. Physiological responses to prolonged bed rest and fluid immersion in man: A compendium of research (1974-1980). NASA Technical Memorandum 81324, Moffett Field, CA, p1-110, 1982

Greenleaf JE, Wade CE, and Leftheriotis G. Orthostatic responses following 30-day bedrest deconditioning with isotonic and isokinetic exercise trainig. Aviat Space Environ Med 60:537-542, 1989

Gregoreva TA. The Innervation of Blood Vessels. Pergamon, New York, 1962

Guo GB and Abboud FM. Angiotensin II attenuates the baroreflex control of heart rate and sympathetic nerve activity. Am J Physiol 246 (Heart Circ Physiol 15): H80-H89, 1984

Gupta PD. Spinal autonomic afferents in elicitation of tachycardia in volume infusion in the dog. Am J Physiol 229:303-308, 1975

Guttmann L, Munro AF, Robinson R, and Walsh JJ. Effect of tilting on the cardiovascular responses and plasma catecholamine levels in spinal man. Paraplegia 1:4-18, 1963

Guyton AC, Coleman TG, Cowley AW, Manning RD, Norman RA, and Ferguson JD. A systems analysis approach to understanding long-range arterial blood pressure control and hypertension. Circ Res 35:159-176, 1974

Guz AM, Noble IM, Widdicombe JG, Threnchard D, Muschin WW, and Makey AR. The role of vagal and glossopharyngeal afferent nerves in respiratory sensation, control of breathing and arterial pressure regulation in conscious man. Clin Sci 30:161-170, 1966

Hagbarth K-E and Vallbo AB. Pulse and respiratory grouping of sympathetic impulses in human muscle nerves. Acta Physiol Scand 74: 96-108, 1968

Hales JRS and Ludbrook J. Baroreflex participation in redistribution of cardiac output at onset of exercise. J Appl Physiol 64:627-634, 1988

Hamilton WF, Woodbury RA, and Harper HT, Jr. Physiologic relationships between intrathoracic, intraspinal and arterial pressures. J Am Med Assoc 107:853-856, 1936

Harper CM and Lyles YM. Physiology and complications of bed rest. J Am Geriatr Soc 36:1047-1054, 1988

Harrison MH, Rittenhouse D, and Greenleaf JE. Effect of posture on arterial baroreflex control of heart rate in humans. Eur J Appl Physiol Occup Physiol 55:367-373, 1986

Hasser EM, DiCarlo SE, Applegate RJ, and Bishop VS. Osmotically released vasopressin augments cardiopulmonary reflex inhibition of the circulation. Am J Physiol 254 (Regulatory Integrative Comp Physiol 23): R815-R820, 1988

Hasser EM, Undesser KP, and Bishop VS. Interaction of vasopressin with area postrema during volume expansion. Am J Physiol 253 (Regulatory Integrative Comp Physiol 22): R605-R610, 1987

Hauss WH, Kreuziger H, and Asteroth H. Uber die Reizung der Pressorezeptoren im Sinus caroticus beim Hund. Z Kreislaufforsch 38:28-33, 1949

Hayward L, Hay M, and Felder RB. Acute resetting of the carotid sinus baroreflex by aortic depressor nerve stimulation. Am J Physiol 264 (Heart Circ Physiol 33): H1215-H1222, 1993

Heath GW, Gavin JR, Hinderliter JM, Hagberg JM, Bloomfield SA, and Hollozy JO. Effects of exercise and lack of exercise on glucose tolerance and insulin sensitivity. J Appl Physiol 54:512-517, 1983

Heesch CM, Abboud FM, and Thames MD. Acute resetting of carotid sinus baroreceptors I. Dissociation between discharge and wall changes. Am J Physiol 247 (Heart Circ Physiol 16): H824-H832, 1984a

Heesch CM, Abboud FM, and Thames MD. Acute resetting of carotid sinus baroreceptors II. Possible involvement of electrogenic Na^+ pump. Am J Physiol 247 (Heart Circ Physiol 16): H833-H839, 1984b

Heesch CM and Carey LA. Acute resetting of arterial baroreflexes in hypertensive rats. Am J Physiol 253 (Heart Circ Physiol 22): H974-H979, 1987

Hilsted J. Pathology in diabetic autonomic neuropathy: cardiovascular, hormonal and metabolic studies. Diabetes 31:730-737, 1982

Hodgkin AL and Katz B. The effect of sodium ions on the electrical activity of the giant squid axon. J Physiol Lond 108:37-77, 1949

Hoffler GW. Cardiovascular studies of US space crews: an overview and perspective. In: Cardiovascular Flow Dynamics and Measurements, edited by NHC Hwang and NA Normann, University Park Press, Baltimore, MD, p335-363, 1977

Holmes AE and Ledsome JR. Effect of norepinephrine and vasopressin on carotid sinus baroreceptor activity in the anesthetized rabbit. Experientia Basel 40:825-827, 1984

Hordinsky JR, Gebhart W, Wegmann GHM, and Shafer G. Cardiovascular and biochemical responses to simulated flight entry. Aviat Space Environ Med 52:16-18, 1981

Hubbard SJ. A study of rapid mechanical events in a mechanoreceptor. J Physiol Lond 141:198-218, 1958

Hyatt KH. Hemodynamic and body fluid alterations induced by bedrest. In: Hypogravic and Hypodynamic Environments, edited by RH Murray and M McCally, NASA SP-269, p189-209, 1971

Hyatt KH, Jacobson LB, and Schneider VS. Comparison of 70° tilt, LBNP, and passive standing as a measure of orthostatic tolerance. Aviat Space Environ Med 46:801-806, 1975

Hyatt KH, Kamenetsky LG, and Smith WM. Extravascular dehydration as an etiologic factor in post-recumbancy orthostatism. Aerospace Med 40:644-650, 1969

Hyatt KH and West DA. Reversal of bedrest-induced orthostatic tolerance by lower body negative pressure and saline. Aviat Space Environ Med 48:120-124, 1977

Igler FO, Donegan JH, Hoo KC, Korns ME, and Kampine JP. Chronic localized hypotension and resetting of carotid sinus baroreceptors. Circ Res 49:649-654, 1981

Imaizumi T, Takeshita A, Higashi H, and Nakamura M. Alpha-ANP alters reflex control of lumbar and renal sympathetic nerve activity and heart rate. Am J Physiol 253 (Heart Circ Physiol 22): H1136-H1140, 1987

Imam K, Maddens M, Mohanty PK, Felicetta JV, and Sowers JR. Atrial natriuretic peptide attenuates the reflex sympathetic responses to lower body negative pressure. Am J Med Sci 298:1-7, 1989

Imholz BPM, Van Montfrans GA, Settels JJ, Van Der Hoeven GMA, Karemaker JM, and Weiling W. Continuous non-invasive blood pressure monitoring: Reliability of Finapres device during the Valsalva manoeuvre. Cardiovasc Res 22:390-397, 1988

Ioffe LA. Reaction of the heart to orthostatic effects. Kardiologia 1:116-124, 1968

Johnson JM, Rowell LB, Niederberger M, and Eisman MM. Human splanchnic and forearm vasoconstrictor responses to reductions of right atrial and aortic pressures. Circ Res 34:515-524, 1974

Johnson PC, Driscoll TB, and LeBlance AD. Blood volume changes. In: Biomedical Results from Skylab, edited by RS Johnston and LF Dietlein, NASA SP-377, p27-45, 1977a

Johnson RL, Hoffler GW, Nicogossian A, Bergman SA, and Jackson MM. Lower body negative pressure: Third manned Skylab mission. In: Biomedical Results from Skylab, edited by RS Johnston and LF Dietlein, NASA SP-377, p284-312, 1977b

Jose AD and Taylor RR. Autonomic blockade by propranolol and atropine to study intrinsic myocardial function in man. J Clin Invest 48:2029-2031, 1969

Kasting GA, Eckberg DL, Fritsch JM, and Birkett CL. Continuous resetting of the human carotid baroreceptor-cardiac reflex. Am J Physiol 252 (Regulatory Integrative Comp Physiol 21): R732-R736, 1987

Katona PG, Poitras JW, Barnett GD, and Terry BS. Cardiac vagal efferent activity and heart period in the carotid sinus reflex. Am J Physiol 218:1030-1037, 1970

Kedzi P. Control by the superior cervical ganglion of the state of contraction and pulsatile expansion of the carotid sinus arterial wall. Circ Res 2:367-371, 1954

Kiowski W and Julius S. Renin response to stimulation of cardiopulmonary mechanoreceptors in man. J Clin Invest 62:656-663, 1978

Koch E. Die reflektorische selbsteuerung des kreislaufes. In: Ergebnisse der Kreislaufforschung, edited by B. Kirsch, Steinkopff, Dresden, p1-234, 1931

Korner PI, Tonkin AM, and Uther JB. Reflex and mechanical circulatory effects of graded Valsalva maneuvers in normal man. J Appl Physiol 40:434-440, 1976

Koushanpour E. Baroreceptor discharge behavior and resetting. In: Baroreceptor Reflexes, edited by: PB Persson and HR Kirchheim, Springer-Verlag, New York, p9-44, 1991

Kreiger EM. Time course of baroreceptor resetting in acute hypertension. Am J Physiol 218:486-490, 1970

Kunze DL. Rapid resetting of the carotid baroreceptor reflex in the cat. Am J Physiol 241 (Heart Circ Physiol 10): H802-H806, 1981

Kunze DL and Brown AM. Sodium sensitivity of baroreceptors and fluid volume in the cat. Circ Res 42:714-720, 1978

Kunze DL, Krauhs JM, and Orlea CJ. Direct action of norepinephrine on aortic baroreceptors of rat adventitia. Am J Physiol 247:H811-H816, 1984

Kunze DL, Saum WR, and Brown AM. Sodium sensitivity of baroreceptor mediates reflex changes of blood pressure and urine flow. Nature 267:75-78, 1977

Lamb LE, Johnson RL, Stevens PM, and Welch BE. Cardiovascular deconditioning from space cabin simulator confinement. Aerospace Med 35:420-428, 1964

Lamb LE, Stevens PM, and Johnson RL. Hypokinesia secondary to chair rest from 4 to 10 days. Aerospace Med 36:755-763, 1965

Lancaster MS and Triebwasser JR. The effects of total body exercise on the metabolic, hematologic and cardiovascular consequences of prolonged bedrest. In: Hypogravic and Hypodynamic Environments, edited by RH Murray and M McCally, NASA SP-369, p225-248, 1971

Leach CS and Jonhson PC. Influence of space flight on erythrokinetics in man. Science 225:216-218, 1984

Ledsome JR and Kan W. Reflex changes in hindlimb and renal vascular resistance in response to distension of the isolated pulmonary arteries of the dog. Circ Res 40:64-72, 1977

Lee WB and Lumbers ER. Angiotensin and the cardiac baroreflex response to phenylephrine. Clin Exp Pharmacol Physiol 8:109-117, 1981

Leon DF, Shaver JA, and Leonard JJ. Reflex heart rate control in man. Am Heart J 80:729-739, 1970

Lippold OCJ, Nicholls JG, and Redfearn JWT. Electrical and mechanical factors in the adaptation of mammalian muscle spindle. J Physiol Lond 153:209-217, 1960

Ludwig DA and Convertino VA. A statistical note on the redundancy of nine standard baroreflex parameters. Aviat Space Environ Med 62:172-175, 1991

Luft UC, Myhre LC, Loeppky JA, and Venters MD. A Study of Factors Affecting Tolerance to Gravitational Stress Simulated by Lower Body Negative Pressure. Lovelace Foundation, (NASA contract NAS9-14472), Albuquerque, NM, 1-60, 1976

Ludbrook J and Graham WF. The role of cardiac receptor and atrial baroreceptor reflexes in control of the circulation during acute changes in blood volume in conscious rabbit. Circ Res 54:424-435, 1984

Ludbrook J, Mancia G, Ferrari A, and Zanchetti A. Factors influencing the carotid baroreceptor response to pressure changes in a neck chamber. Clin Sci Mol Med 51:347s-349s, 1977

Lumbers ER, McCloskey DI, and Potter EK. Inhibition by angiotensin II of baroreceptor-evoked activity in cardiac vagal efferent nerves in the dog. J Physiol Lond 294:69-80, 1979

Mack GW, Shi X, Nose H, Tripathi A, and Nadel ER. Diminished baroreflex control of forearm vascular resistance in physically fit humans. J Appl Physiol 63:105-110, 1987

Malliani A, Lombardi F, Pagani M, Recordati G, and Schwartz RJ. Spinal cardiovascular reflexes. Brain Res 87:239-246, 1975

Mancia G, Bonazzi O, Pozzoni L, Ferrari A, Gardumi M, Gregorini L, and Perondi R. Baroreceptor control of atrioventricular conduction in man. Circ Res 44:752-758, 1979

Mancia G, Ferrari A, Gregorini L, Valentini R, Ludbrook J and Zanchetti A. Circulatory reflexes from carotid and extra carotid baroreceptor areas in man. Circ Res 41:309-315, 1977

Mancia G and Mark AL. Arterial baroreflexes in humans. In: Handbook of Physiology: The Cardiovascular System. Peripheral Circulation and Organ Blood Flow, part. 2, vol. 3, edited by Shepherd JT and Abboud FM, American Physiological Society, Bethesda, MD, p755-793, 1983

Mark AL and Mancia G. Cardiopulmonary baroreflexes in humans. In: Handbook of Physiology: The Cardiovascular System. Peripheral Circulation and Organ Blood Flow, part. 2, vol. 3, edited by Shepherd JT and Abboud FM, American Physiological Society, Bethesda, MD, p795-813, 1983

Marks ES, Ohman KP, andd Keiser HR. Atrial natriuretic factor enhances volume-and pressor-induced heart rate response in the conscious rat. Acta Physiol Scand 138:161-165, 1990

Matsukawa S and Reid IA. Role of the area postrema in the modulation of the baroreflex control of heart rate by angiotensin II. Circ Res 67:1462-1473, 1990

Matsumura Y, Hasser EM, and Bishop VS. Central effect of angiotensin II on baroreflex regulation in conscious rabbits. Am J Physiol 256 (Regulatory Integrative Comp Physiol 25): R694-R700, 1989

McCally M, Piemme TE, and Murray RH. Tilt table responses of human subjects following application of lower body negative pressure. Aerospace Med 37:1248-1249, 1966

McCally M, Pohl SA, and Sampson PA. Relative effectiveness of selected spaceflight deconditioning countermeasures. Aerospace Med 39:722-734, 1968

McCally M and Wunder CC. Immersion techniques and the evaluation of spaceflight deconditoning countermeasures. In: Hypogravic and Hypodynamic Environments, edited by RH Murray and M McCally, NASA SP-369, p323-344, 1971

McCubbin JW, Green JH, and Page IH. Baroreceptor function in chronic renal hypertension. Circ Res 4:205-210, 1956

Melada GA, Goldman RH, Leutscher JA, and Zager PG. Hemodynamic, renal function, plasma renin and aldosterone in man after 5 to 14 days of bed rest. Aviat Space Environ Med 46:1049-1055, 1975

Mendelsohn FA and Johnston CI. Renin release in chronic paraplegia. Aust N Z J Med 4:393-397, 1971

Mendelowitz D. and Scher AM. Pulsatile sinus pressure changes evoke sustained baroreflex responses in awake dogs. Am J Physiol 255 (Heart Circ Physiol 24): H673-H678, 1988

Mifflin SW and Kunze DL. Rapid resetting of low pressure vagal receptors in the superior vena cava of the rat. Circ Res 51:241-249, 1982

Miller PB, Hartman BO, Johnson RL, and Lamb LE. Modification of the effects of two weeks of bedrest upon circulatory function in man. Aerospace Med 35:931-939, 1964a

Miller PB, Johnson RL, and Lamb LE. Effects of four weeks of absolute bed rest on circulatory functions in man. Aerospace Med 35:1194-1200, 1964b

Morgan DA, Balon TW, and Mark AL. Hyperinsulinemia produces exaggerated increase in sympathoadrenal activity in spontaneously hypertensive rats. Hypertension 16:339, 1990

Mroczek WJ, Lee WR, David ME, and Finnerty FA. Vasodialator administration in the presence of beta adrenergic blockade. Circulation 53:985-988, 1976

Munch PA, Andresen MC, and Brown AM. Rapid resetting of aortic baroreceptors in vitro. Am J Physiol 244 (Heart Circ Physiol 13): H672-680, 1983

Munch PA and Brown AM. Role of vessel wall in acute resetting of aortic baroeceptors. Am J Physiol 248 (Heart Circ Physiol 17):H843-H852, 1985

Munch PA, Thoren PN, and Brown AM. Dual effects of norepinephrine and mechanisms of baroreceptor stimulation. Circ Res 61:409-419, 1987

Muratori G. Histological observations on the structure of the carotid sinus in man and mammals. In: Baroreceptors and Hypretension, edited by P. Kedzi, Pergamon Press, Oxford, p253-265, 1967

Musgrave FS, Zechman FW, and Main RS. Changes in total leg volume during lower body negative pressure. Aerospace Med 40:602-606, 1969

Myers HA and Honig CR. Influence of initial resistance on magnitude of response of vasomotor stimuli. Am J Physiol 216:1429-1436, 1969

Nakamaru M and Inagami T. Atrial natriuretic peptide inhibits norepinephrine release evoked by sympathetic nerve stimulation in isolated perfused rat mesenteric arteries. Eur J Pharmacol 123:459-461, 1986

Nakayama S. The circulatory and respiratory reflexes from the subclavian and brachiocephalic artery. Japan J Physiol 15:290-293, 1965

Nicogossian AE, Charles JB, Bungo MW, and Leach-Huntoon CS. Cardiovascular function in space flight. Acta Astronautica 24:323-328, 1991

Niijima, A. The effect of ANP on the efferent activity of autonomic nerves. Arch Histol Cytol 52:325-329, 1989

Nonindez JF. The aortic (depressor) nerve and its associated epitheloid body, the glomus aorticum. Am J Anat 57:259-301, 1935

O'Leary DS. Autonomic mechanisms of muscle metaboreflex control of heart rate. J Appl Physiol 74:1748-1754, 1993

Paintal AS. A study of right and left atrial receptors. J Physiol Lond 120:596-610, 1953

Pawelczyk JA and Raven PB. Reductions in central venous pressure improve carotid baroreflex responses in conscious men. Am J Physiol 257 (Heart Circ Physiol):H1389-H1395, 1989

Pestov ID and Geratewohl SJ. Weightlessness. In: Foundations of Space Biology and Medicine. Ecological and Physiological Bases of Space Biology and Medicine, vol. 2, book 1, edited by M Calvin and OG Gazenko, NASA, Washington, DC, p305-354, 1975

Persson PB. Interaction of Arterial and Cardiopulmonary Reflexes. In: Baroreceptor Reflexes, edited by: PB Persson and HR Kirchheim, Springer-Verlag, New York, p126-153, 1991

Pickering TG, Gribbin B, Petersen ES, Cunningham DJC, and Sleight P. Comparison of the effects of exercise and posture on the baroreflex in man. Cardiovasc Res 5:582-586, 1971

Pickering TG, Gribbin B, Strange-Petersen E, Cunningham DJC, and Sleight P. Effects of autonomic blockade on the baroreflex in man at rest and during exercise. Circ Res 30:177-185, 1972

Poliner LR, Dehmer GJ, Lewis SE, Parkey RW, Blomqvist CG, and Willerson JT. Left ventricular performance in normal subjects: A comparison of the response to exercise in the upright and supine positions. Circulation 62:528-534, 1980

Poole CJM, Williams TDM, Lightman SL, and Frankel HL. Neuroendocrine control of vasopressin secretion and its effect on blood pressure in subjects with spinal cord transection. Brain 110:727-735, 1987

Porth JM, Bamrah VS, Tristiani FE, and Smith JE. The Valsalva maneuver: mechanisms and clinical implications. Heart and Lung 13:507-518, 1984

Raven PB, Rohm-Young D and Blomqvist CG. Physical fitness and cardiovascular response to lower body negative pressure. J Appl Physiol 56:138-144, 1984

Rees PM. The distribution of biogenic amines in the carotid bifurcation region. J Physiol 193:245-253, 1967

Rees PM. Electron microscopical observations on the architecture of the carotid sinus walls, with special reference to the sinus portion. J Anat 103:35-47, 1968

Rees PM and Jepson P. Measurement of arterial geometry and wall composition in the carotid sinus baroreceptor area. Circ Res 26:461-467, 1970

Reid IA. Actions of angiotensin II on the brain: mechanisms and physiologic role. Am J Physiol 246 (Renal Fluid Electrolyte Physiol 15): F533-F543, 1984

Reis DJ and Fuxe K. Adrenergic innervation of the carotid sinus. Am J Physiol 215:1054-1057, 1968

Robinson BF, Epstein SE, Beiser GD, and Braunwald E. Control of heart rate by the autonomic nervous system. Studies in man on the interrelations between baroreceptor mechanisms and exercise. Circ Res 19:400-411, 1966

Roddie IC and Shepherd JT. The reflex nervous control of human skeletal muscle blood vessels. Clin Sci 15:433-440, 1956

Roddie IC and Shepherd JT. The effects of carotid arterty compression in man with special reference to changes in vascular resistance in the limbs. J Physiol Lond 139:377-384, 1957

Roddie IC and Shepherd JT. Receptors in the high pressure and low pressure vascular systems: their role in the reflex control of the human circulation. Lancet 1:493-496, 1958

Roddie IC, Shepherd JT, and Whelan RF. Reflex changes in vasoconstrictor tone in human skeletal muscle in response to stimulation of receptors in a low-pressure area of the intrathoracic bed. J Physiol Lond 139:369-376, 1957

Rowell LB. Human Circulation: Regulation During Physical Stress. Oxford University Press, New York, 1986

Sakaguchi T, Takahashi M, and Bray GA. Lateral hypothalamus and sympathetic firing rate. Am J Physiol 255 (Regulatory Integrative Comp Physiol 24): R507-R512, 1988

Salgado HC and Kreiger EM. Time course of baroreceptor resetting in short term hypotension in the rat. Am J Physiol 234 (Heart Circ Physiol.3): H552-H556, 1978

Saltin B, Blomqvist CG, Mitchell JN, Johnson RL, Wildenthal K, and Chapman CB. Response to exercise after bed rest and after training. Circulation 38(suppl 7):1-78, 1968

Samueloff SL, Browse NL, and Shepherd JT. Response of capacity vessels in human limbs to head-up tilt and suction on lower body. J Appl Physiol 21:47-54, 1966

Sanders JS, Ferguson DW, and Mark AL. Arterial baroreflex control of sympathetic nerve activity during elevation of blood pressure in normal man: Dominance of aortic baroreflexes. Circulation 77:279-288, 1988

Sandler H. Cardiovascular effects of inactivity. In: Inactivity: Physiological Effects, edited by H Sandler and J Vernikos, Academic Press, Orlando, FL, p1-9, 1986

Sandler H. Effects of bed rest and weightlessness on the heart. In: Hearts and Heart-Like Organs, edited by GH Bourne, Academic Press, New York, p435-524, 1980

Sandler H. Low-G simulation in mammalian research. Physiologist 22:S19-S24, 1979

Sandler H, Goldwater DJ, Popp RL, Spaccavento L, and Harrison DC. Beta-blockade in the compensation for bedrest cardiovascular deconditioning: physiological and pharmacological observations. Am J Cardiol 55:114D-120D, 1985

Sandler H and Winter DL. Physiological responses of women to simulated weightlessness: A review of the significant findings of the first female bedrest study. NASA SP-430, p1-187, 1978

Sandroni P, Benarroch EF, and Low PA. Pharmacological dissection of components of the Valsalva maneuver in adrenergic failure. J Appl Physiol 71:1563-1567, 1991

Sarnoff S, Hardenbergh JE, and Whittenberger JL. Mechanism of the arterial pressure response to the Valsalva test: the basis for its use as an indciator of the intactness of the sympathetic outflow. Am J Physiol 154: 316-327, 1948

Sather TM, Goldwater DJ, Montgomery LD, and Convertino VA. Cardiovascular dynamics associated with tolerance to lower body negative pressure. Aviat Space Environ Med 57:413-419, 1986

Scher AM and Ito CS. Regulation of arterial blood pressure by aortic baroreceptors in the unanesthetized dog. Circ Res 42:230-236, 1978

Seals DR and Chase PB. Influence of physical training on heart rate variability and baroreflex circulatory control. J Appl Physiol 66:1886-1895, 1989

Sharpey-Schafer EP. Effect of respiratory acts on the circulation. In: Handbook of Physiology: Circulation, part. 2, vol. 3, edited by Hamilton FW and Dow P, American Physiological Society, Washington, DC, p1875-1886, 1965

Shepherd JT and Mancia G. Reflex control of the human cardiovascular system. Rev Physiol Biochem Pharmacol 105:1-99, 1986

Shvartz E, Convertino VA, Keil LC, and Haines RF. Orthostatic fluid-electrolyte and endocrine responses in fainters and non-fainters. J Appl Physiol 51:1404-1410, 1981

Simon ACH, Safar ME, Weiss YA, London JM, and Milliez PL. Baroreflex sensitivity and cardiopulmonary blood volume in normotensive and hypertensive patients. Br Heart J 39:799-805, 1977

Sleight P, Robinson JL, Brooks DE, and Rees PM. Characteristics of single baroreceptor fibers and whole nerve activity in the normotensive and renal hypertensive dog. Circ Res 41:750-758, 1977

Smith JJ, Hughes CV, Ptacin MJ, Barney JA, Tristiani FE, and Ebert TJ. The effect of age on the hemodynamic response to graded postural stress in normal men. J Gerontol 42:406-411, 1987a

Smith ML and Raven PB. Cardiovascular responses to lower body negative pressure in endurance and static exercise-trained men. Med Sci Sports Exer 18:545-550, 1986

Smith ML, Graitzer HM, Hudson DL, and Raven PB. Baroreflex function in endurance and static exercise trained men. J Appl Physiol 64:585-591, 1988

Smith SA, Stallard TJ, Salih MM, and Littler WA. Can sinoartoic baroreceptor heart rate reflex sensitivity be determined from phase IV of the Valsalva maneuver? Cardiovascular Res 21:422-427, 1987b

Somers VK, Conway J, LeWinter M, and Sleight P. The role of baroreflex sensitivity in post-exercise hypotension. J Hypertens 3:S129-S130, 1985

Sprenkle JM, Eckberg DL, Goble RG, Schelhorn JJ, and Halliday HC. Device for rapid quantification of human carotid baroreceptor-cardiac reflex responses. J Appl Physiol 60:727-732, 1986

Stegemann J, Baer FM, and Hoffman U. The Valsalva maneuver as an indirect, non-invasive indicator of central blood volume shift. Aviat Space Environ Med 9:422-427, 1985

Stegemann J, Busert A, and Brock D. Influence of fitness on the blood pressure control system in man. Aerospace Med 45:45-48, 1974

Stegemann J, Meier U, Skipka W, Hartlieb W, Hemme B, and Tibes U. Effects of multi-hour immersion with intermittent exercise on urinary excretion and tilt table tolerance in athletes and nonathletes. Aviat Space Environ Med 46:26-29, 1975

Stevens PM and Lamb LE. Effects of lower body negative pressure on the cardiovascular system. Am J Cardiol 16:506-516, 1965

Stevens PM, Miller PB, Gilbert CA, Lynch TN, Johnson RL, and Lamb LE. Influence of long-term lower body negative pressure on the circulatory function of man during prolonged bed rest. Aerospace Med 37:357-367, 1966

Stinnett HO, Bishop VS, and Peterson DF. Reduction in baroreflex cardiovascular responses due to venous infusion in the rabbit. Circ Res 39:766-772, 1976

Takeshita A, Mark AL, Eckberg DL, and Abboud FM. Effect of central venous pressure on arterial baroreflex control of heart rate. Am J Physiol 236 (Heart Circ Physiol) 5:H42-H47, 1979

Tan W and Zucker IH. Acute carotid baroreflex resetting in conscious dog. J Physiol Lond 416:557-569, 1989

Tatro DL, Dudley GA, and Convertino VA. Carotid-cardiac baroreflex response and LBNP tolerance following resistance training. Med Sci Sports Exerc 24:789-796, 1992

Ten Harkel, ADJ, Van Lieshout JJ, Van Lieshout EJ, and Wieling W. Assessment of cardiovascular reflexes: influence of posture and period of preceeding rest. J Appl Physiol 8:147-153, 1990

TerKonda SP and Bishop VS. Role of atrial natriuretic factor on the baroreflex control of renal sympathetic nerve activity in the conscious rabbit. Circ 76 [Suppl Part II]:IV-348, 1987

Thompson CA, Tatro DL, Ludwig DA, and Convertino VA. Baroreflex responses to acute changes in blood volume in humans. Am J Physiol 259 (Regulatory Integrative Comp Physiol 28): R792-R798, 1990

Thoren PN, Donald DE, and Shepherd JT. Role of heart and lung receptors with non-medullated vagal afferents in circulatory control. Circ Res 38:II-2-II-9, 1976

Thorn PN, Donald DE, and Shepherd JT. Role of heart and lung receptors with nonmedulated vagal afferents in circulatory control. Circ Res 38 Suppl II: II112-II119, 1976.

Thorton WE and Hoffler GW. Hemodynamic study of the legs under weightlessness. In: Biomedical Results from Skylab, edited by RS Johnston and LF Dietlein, NASA SP-377, p324-329, 1977

Tomomatsu E and Nishi K. Increased activity of carotid sinus baroreceptors by sympathetic stimulation and norepinephrine. Am J Physiol 240:H650-658, 1981

Touw K, Fink G, Haywood JR, Shaffer RA, and Brody MJ. Elevated neurogenic vasoconstrictor tone is the mechanism of hypertension in rats with aortic baroreceptor reafferentation (abstract). Fed Proc 38:1232, 1979

Undesser KP, Hasser EM, Haywood JR, Johnson AK, and Bishop VS. Interactions of vasopressin with the area postrema in arterial baroreflex function in conscious rabbits. Circ Res 56:410-417, 1985

Undesser KP, Jing-Yun P, Lynn MP, and Bishop VS. Baroreflex control of sympathetic nerve activity after elevations of pressure in conscious rabbits. Am J Physiol 248 (Heart Circ Physiol 17): H827-H834, 1975

Undesser KP, Lynn MP, and Bishop VS. Rapid resetting of aortic nerves in conscious rabbits. Am J Physiol 246 (Heart Circ Physiol 15): H302-H305, 1984

Vatner SF, Boettcher DH, Heyndrickx GR, and McRitchie RJ. Reduced baroreflex sensitivity with volume loading in conscious dogs. Circ Res 37:236-242, 1975

Vogt FB. Tilt table and plasma volume changes with short term deconditioning exposure. Aerospace Med 38:564-568, 1967

Volpe M, Cuocolo A, Vecchione F, Mele AF, Condorelli M, and Trimarco B. Vagal mediation of the effects of atrial natriuretic factor on blood pressure and arterial baroreflexes in the rabbit. Circ Res 60:747-755, 1987

Wallin BG, Sundlof G, and Delius W. The effect of carotid sinus nerve stimulation on muscle and skin nerve sympathetic activity in man. Pflugers Arch 358:101-110, 1975

Wang W, Weitzel MA, Chen J-S, and Zucker IH. Acute volume expansion enhances baroreceptor discharge in dogs. Am J Physiol 262 (Heart Circ Physiol 31): H209-H214, 1992

Werber AH and Fink GD. Aortic baroreceptor denervation in the rat as a model for borderline hypertension in the human (abstract). Fed Proc 38:1232, 1979

White RJ, Leonard RS, Srinivasan RB, and Charles JB. Mathematical modeling of acute and chronic cardiovascular changes during extended duration orbiter (EDO) flights. Acta Astronautica 23:41-51, 1991

Whitney RJ. The measurement of volume changes in human limbs. J Physiol Lond 121:1-27, 1953

Wolthuis RA, Bergman SA, and Nicogossian AE. Physiological effects of locally applied reduced pressure in man. Physiol Rev 54:566-595, 1974

Wurster RD and Trobiani S. Effects of cervical sympathetic stimulation on carotid occlusion reflexes in cats. Am J Physiol 225:978-981, 1973

Zoller RP, Mark AL, Abboud FM, Schmid PG, and Heistad DD. The role of low pressure baroreceptors in reflex vasoconstrictor responses in man. J Clin Invest 51:2957-2972, 1972

BIOGRAPHICAL SKETCH

Keith A. Engelke graduated from Shawnee High School in Medford, New Jersey, in 1979. He received the Bachelor of Science degree from Wake Forest University in 1983 and the Master of Science degree from the University of Arizona in 1985. After teaching at the University of Arizona (1985-1987) and Pepperdine University (1987-1990), he returned to graduate school in the fall of 1990. During his tenure as a doctoral student, he was supported by a Florida Space Grant Consortium-Space Grant Fellowship and was actively involved in research projects conducted at NASA-Kennedy Space Center, NASA-Ames Research Center, and the Laboratory for Aerospace Cardiovascular Research at Brooks Air Force Base. Following completion of his degree, he plans to continue his career in research and education.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Charles E. Wood, Chair
Professor of Physiology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Victor A. Convertino, Cochair
Associate Professor of
Exercise and Sport Sciences

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Scott K. Powers
Professor of Exercise and
Sport Sciences

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Thomas J. Wronski
Associate Professor of
Veterinary Medicine

This dissertation was submitted to the Graduate Faculty of the College of Medicine and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

April, 1994

Mohan K Raizada
Dean, College of Medicine

Karen G. Holbrook
Dean, Graduate School

UNIVERSITY OF FLORIDA
3 1262 08554 3188

UNIVERSITY OF FLORIDA
3 1262 08554 3188